PARASITOLOGY

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(Founded by G. H. F. Nuttall, F.R.S.)

EDITED BY

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VOLUME 49, 1959

CAMBRIDGE
AT THE UNIVERSITY PRESS
1959

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)
and published by the Cambridge University Press
Bentley House, 200 Euston Road, London, N.W. 1
American Branch: 32 East 57th Street, New York 22, N.Y.
Agent in Canada: The Macmillan Company of Canada Limited

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HENNEGUYA TEGIDIENSIS SP.NOV. (MYXOSPORIDIA), FROM THE FRESHWATER FISH *COREGONUS CLUPEOIDES PENNANTII* (THE GWYNIAD)

BY W. L. NICHOLAS AND J. W. JONES

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(With Plate I and 1 Figure in the Text)

A parasitic protozoan, which we believe to be a previously undescribed species of *Henneguya* Thelophan, 1895, emend. Davis, 1944, is a common cause of disease in the freshwater fish *Coregonus clupeoides pennantii* Cuvier & Valenciennes. *C. pennantii*, known as the gwyniad or white-fish, has only been recorded from Llyn Tegid (Lake Bala) and, rarely, the River Dee. Closely related species are found in various parts of Europe and North America.

In the course of a study of the gwyniad by one of us (J. W. J.), swellings were found on their bodies, which on closer inspection proved to be myxosporidian cysts, which develop in the muscle of infected fish.

THE CYSTS

When they first become visible as swellings on the surface of the fish, the cysts are spherical and about 2 mm. in diameter. They may become much larger, often assuming an elliptical form, and have been found measuring up to 2 cm. in length and 1 cm. in width. A number of cysts are often present on one fish and may occur anywhere in the somatic musculature, but we have not found them elsewhere in the fish. Sometimes ruptured cysts have been found, their contents having been released into the surrounding tissues, but perhaps this has been the result of rough handling the fish received while being removed from the gill-net with which they are caught.

The cysts, which can easily be dissected out from the surrounding tissues (Pl. I, fig. 1), consist of a fibrous capsule, undoubtedly produced by the fish, lined by syncitial protoplasm belonging to the parasite. The syncitium is densely nucleated and from it developing pansporoblasts form a distinct zone next to the syncitial layer, the more mature pansporoblasts and the spores which develop within them moving into the lumen of the cyst as they develop. The lumen of the cyst, which is filled with fluid, contains a suspension of mature spores which appears milky to the naked eye. Parts of histological sections through cysts are shown in Pl. I, figs. 2, 3. Earlier stages in the development of the parasite within the fish have not been found.

Similar cysts are produced in the muscles of fish by other species of *Henneguya*, e.g. *H. salmonicola*, *H. zschokkei* and *H. nonasa* (Kudo, 1920).

THE SPORE

As has been the general practice among workers on the Myxosporidia, our description of the species will depend primarily on the shape and dimensions of the spore. In describing the spore the nomenclature used by Kudo (1920) will be followed. Other phases in the life-cycle of *Henneguya* have seldom been described in sufficient detail to be of much value in comparing species.

The spores are typical of the genus *Henneguya* Thelophan, 1895, as emended by Davis, 1944. There are two polar capsules; each shell valve has a posterior filament or 'tail'; the spore is biconvex and compressed parallel to the sutural plane, and a vacuole staining with iodine is present. The parasite is histozoic and polysporous.

Measurements of the spores are given in Table 1. The spores were preserved in 5% formalin immediately after removal from the cysts (which may have remained unopened in the fish for up to 24 hr. after capture). It is desirable to fix the spore because otherwise the cyst's contents rapidly undergo putrefaction. Like Fish (1939), we found that 5% formalin causes little distortion in the spore, but the vacuole no longer stains with iodine after prolonged storage.

Table 1. *Measurements of spores of* Henneguya tegidiensis

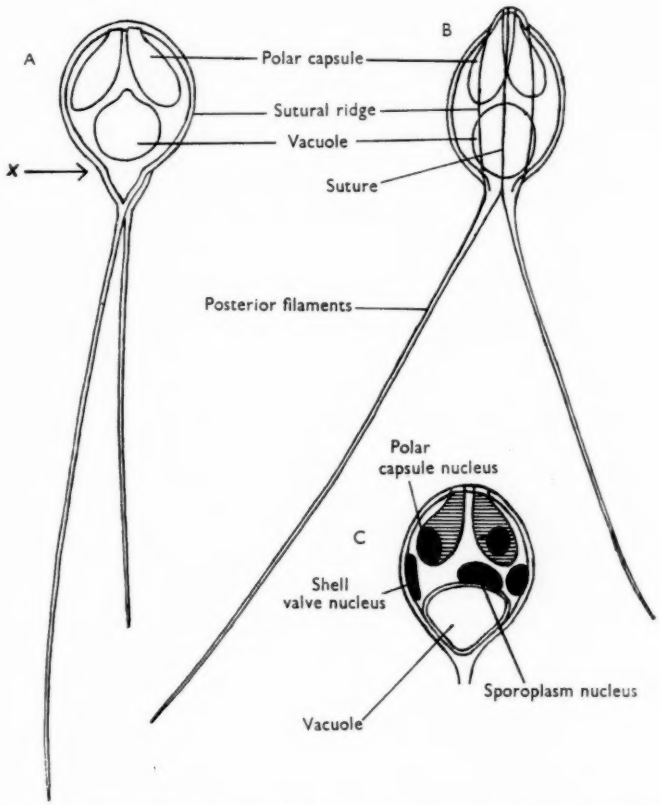
(The spores were fixed and measured in 5% formalin.)

	Mean (μ)	S.D. (μ)	Range (μ)	No. of spores measured
Length of spore, measured from anterior end to *X* (see Text-fig. 1)	9·8	0·75	8–12	92
Breadth of spore	7·8	0·88	7–10	92
Thickness of spore	6·7	0·47	6–7	30
Length of polar capsules	—	—	4–5	30
Breadth of polar capsules	—	—	2–3	30
Length of longer posterior filament, measured from tip to *X*	38·8	8·8	22–56	92
Length of shorter posterior filament, measured from tip to *X*	30·2	8·7	11–46	92
Breadth of sutural ridge	—	—	About 3	—

The structure of the spore is shown in the accompanying figure (Text-fig. 1) and in Pl. I, figs. 4, 5. The spores can be stained by Giemsa's method, the shell staining pale blue and the nuclei and polar capsules very intensively. Most of the spores taken from the cyst fail to stain in Ehrlich's or Heidenhain's haematoxylin, but a small proportion do: in the latter the nuclei stain deeply and the polar capsules less strongly. Perhaps the spores lose their permeability to the stain when fully ripe. With the exception of the nuclei, and the contents of the polar capsules, the structure of the spore can be satisfactorily seen in aqueous mounts of unstained spores. We have not been able to see clearly the internal structure of the polar capsules but the polar filament, characteristic of the myxosporidian polar capsule, appears as a spirally coiled thread of about five turns.

Kudo (1920) has summarized all the literature of the genus *Henneguya* up to date, and his paper, which includes a description of all the known species, has been most

useful in bringing together a very scattered literature. In a later paper by Kudo (1933) references are given to all the fifty-two species described up to the time of its publication. The later literature has been difficult to cover because it is widely scattered, but we have not succeeded in finding descriptions of any species which cannot be distinguished from the gwyniad parasite by the shape and dimensions of the spore. Most of them can be readily distinguished by the shape of the spore,



Text-fig. 1. Spores of *H. tegidiensis*. A and B, as seen unstained and mounted in 5 % formalin. C, drawing based on a number of spores which had been stained in haematoxylin to show the nuclei.

but there are a number of species which differ only in minor points. Host-restriction is well marked in the Myxosporidia, most species being confined to a single host species or closely related hosts (Davis, 1917), and it is interesting to note that two of the species with similar spores are reported from other European species of *Coregonus*.

The two species of *Henneguya zschokkei* (Gurley) Labbe and *H. kolesnikovi* (Gurley) Labbe are re-described and figured by Kudo (1920). They both differ from *H. tegidiensis* in having posterior tail filaments which are not markedly unequal in

length. The spore of *Henneguya kolesnikovi* also differs in being pointed anteriorly. *H. zschokkei* has been found in intramuscular cysts of *Coregonus fera*, *C. schinzii*, *C. liemalis* and in both muscular and branchial cysts of *C. wartmanni nobilis* and *C. exigus albellus*, while *H. kolesnikovi* was found in the thoracic and intercostal muscles of *Coregonus lavarteus*.

A species in which unequal posterior filaments have been reported is *H. salmonicola* Ward (Fish, 1939). This species has been recorded from intramuscular cysts of the Pacific salmon *Onchorynchus keta* (Ward, 1919), *O. kisutch*, *O. gorbuscha* and *O. tschawytscha* (Fish, 1939). Its spores are similar in shape and size to those of *H. tegidiensis*, from which they differ in having polar capsules of unequal length.

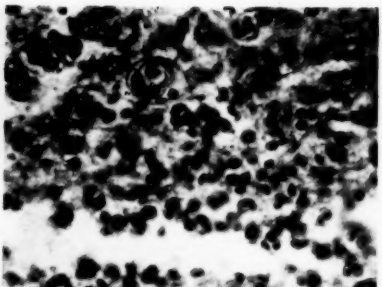
The spores of *H. salmonis* Fantham, Porter & Richardson (1939) also resemble those of the gwyniad parasite rather closely, although the spore body is a little narrower. They differ, however, in their posterior filaments. In *H. salmonis* there is a short undivided posterior appendage, which bifurcates to give rise to the posterior filaments. These may sometimes be slightly unequal in length and may be opposed; they do not taper evenly as do those of other species, but become very thin at a point about half way along their length. *H. salmonis* has been recorded from a single subcutaneous cyst in an Atlantic salmon, *Salmo salar*, from Quebec Province, Canada.

Table 2. *The incidence of cysts of* Henneguya tegidiensis *in gwyniad*, C. clupeoides pennantii, *caught with gill-net in Llyn Tegid* (*Lake Bala*) *North Wales*

Date	No. of fish caught	No. of fish showing cysts	Percentage of fish with cysts
19 July 1955	41	2	4·9
20 July 1955	110	1	0·9
11 October 1955	75	1	1·3
25 October 1955	27	1	3·7
29 December 1955	119	3	2·5
17 January 1956	29	2	6·9
9 March 1956	16	1	6·25
12 April 1956	21	1	4·8
24 May 1956	13	1	7·7
5 July 1956	44	2	4·5
12 July 1956	6	1	16·6
14 August 1956	146	3	2·1
Total	647	19	2·9

We think that the differences between the spores of the gwyniad parasite and those of other described species are sufficient to give this parasite specific status. In view of the high level of host-restriction exhibited by most Myxosporidia, the differences between the spores of this parasite and other species of *Henneguya* taken from *Coregonus*, and to a lesser degree from other Salmonidae, are particularly significant. The part of the fish attacked is most important, but not as significant as differences in the spores, since the same species may apparently attack different parts of the fish, e.g. *H. zschokkei* (see above). The type of lesion produced is not very helpful because similar intramuscular cysts, as noted above, are produced by a number of species of *Henneguya*.

1



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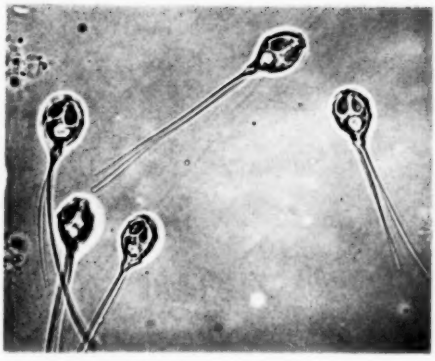

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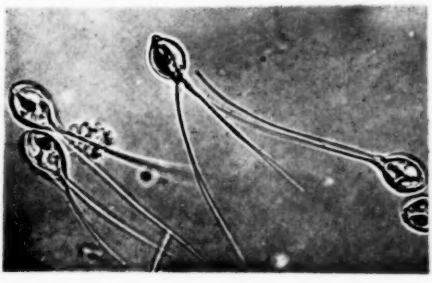

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(*Facing p. 4*)

Since its host, *C. clupeoides pennantii* has a very restricted geographical distribution, it is perhaps appropriate to name the parasite after the lake in which it was found. We therefore propose to call it *Henneguya tegidiensis* sp.nov.

Many Myxosporidia are seasonal in their appearance (Davis, 1917), but we have found no signs of seasonal changes in the incidence of visible cysts in the fish (see Table 2). None of the other species of fish which have been caught in the lake have shown signs of infection. Those which have been caught in numbers are: salmon parr (*Salmo salar*), trout (*S. trutta*), grayling (*Thymallus thymallus*), perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), eel (*Anguilla anguilla*), gudgeon (*Gobio gobio*), and pike (*Esox lucius*).

SUMMARY

A description is given of a new species of *Henneguya* (Myxosporidia), named *H. tegidiensis* sp.nov. It is parasitic in the freshwater fish, *Coregonus clupeoides pennantii*, from Lake Bala (North Wales), producing in it intramuscular cysts.

We wish to thank the Welsh Land Sub-Commission for permission to net the Gwyniad, and Capt. G. H. A. Boyle for his assistance in the netting operation.

REFERENCES

Davis, H. S. (1917). The Myxosporidia of the Beaufort region, a systematic and biological study. *Bull. Bur. Fish.* **35**, 201.

Davis, H. S. (1944). A revision of the genus *Henneguya* (Myxosporidia), with descriptions of two new species. *Trans. Amer. Micr. Soc.* **63**, 311.

Fantham, H. B., Porter, A. & Richardson, L. R. (1939). Some Myxosporidia found in certain freshwater fishes in Quebec province, Canada. *Parasitology*, **31**, 1.

Fish, F. F. (1939). Observations on *Henneguya salmonicola*, a Myxosporidian parasite in Pacific salmon. *J. Parasit.* **25**, 169.

Kudo, R. (1920). Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. *Illinois Biol. Monogr.* **5**, 3–4.

Kudo, R. (1933). A taxonomic consideration of Myxosporidia. *Trans. Amer. Micr. Soc.* **52**, 195.

Ward, H. B. (1919). Notes on North American Myxosporidia. *J. Parasit.* **6**, 49.

EXPLANATION OF PLATE I

Fig. 1. Cysts of *Henneguya tegidiensis* dissected out from the somatic musculature of the fish *Coregonus clupeoides pennantii*.

Fig. 2. Part of a section through a cyst of *H. tegidiensis*, stained in haematoxylin and eosin, × 750. At the bottom of the section part of the fibrous capsule with two fibroblast nuclei can be seen. Lining the capsule there is a heavily nucleated syncitial layer of parasite protoplasm from which developing pansporoblasts are proliferating. Developing and mature spores on the right of the section fill the lumen of the cyst.

Fig. 3. A similar section through a cyst of *H. tegidiensis*, × 375, which includes some of the surrounding fish muscles.

Figs. 4, 5. Spores of *H. tegidiensis* mounted in glycerine jelly, × 750.

(Photographs by W. Irvine)

(*MS. received for publication* 4. xi. 1958.—Ed.)

A REVISION OF THE HYPOASPID MITES ASSOCIATED WITH MYRIAPODA WITH DESCRIPTIONS OF THREE NEW SPECIES OF THE SUBGENUS *JULOLAELAPS* BERL. (ACARINA: LAELAPTIDAE)

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(With 39 Figures in the Text)

The paraphages of Myriapoda belonging to the family Laelaptidae were recently reviewed by Evans (1955). In his paper the author demonstrated the modifications which certain structures undergo during the process of 'adaptation' to a symbiotic mode of life. These are illustrated by reduction which 'may occur in the sclerotization and chaetotaxy of the body, dentition and size of the digits of the chelicerae, chaetotaxy of the gnathosoma and pedipalps, peritreme and peritrematal plate and the ambulacral apparatus'. A study of the new species described below further corroborates these conclusions. It also draws attention to the fact that we must modify our present generic concept of certain members of the group.

The discovery of three new species, new synonymy and the addition of the genera *Scissuralaelaps* Womersley and *Iphiolaelaps* Wom. to the list of laelaptid genera symbiotic on Myriapoda, necessitates a modification of the key given by Evans (1955). *Julolaelaps* is regarded by the present author as a subgenus of the genus *Hypoaspis*. A key to the species of this subgenus is given in the text. A list of the known genera and species occurring on millipedes is given at the end of the paper. The author is indebted to Dr G. O. Evans of the Department of Zoology, British Museum (Nat. Hist.), London, for valuable suggestions and for placing the material on which this paper is based at his disposal.

The type material is deposited in the collections of the British Museum (Nat. Hist.), London.

Revised key to the genera and subgenera of laelaptid paraphages of Myriapoda

1. Sternal shield divided longitudinally — 2
— Sternal shield entire, sclerotized or unsclerotized — 3
2. Stigma without peritreme. — **Iphiolaelaps** Wom.
— Peritreme normal, extending anterior to coxa II. — **Scissuralaelaps** Wom.
3. Claws on leg I absent (or vestigial?), claws on legs II–IV normal; peritreme reduced. — **Iphiopsis** Berl.
— Claws on all legs either normal, absent or vestigial. — 4
4. Only three pairs of gnathosomal setae present. — 5
— Gnathosomal setae normal (4 pairs). — (**Hypoaspis** s.lat. Can.) 7
5. Dorsal shield not completely covering the dorsum of the mite; sternal shield strongly sclerotized; gnathosoma without external posterior rostral setae; anal shield with two setae. — 6

5. Dorsal shield completely covering the dorsum of the mite; without sclerotized sternal shield; gnathosoma without capitular setae; three setae on anal shield **Scolopendracarus** Evans
6. Genital shield markedly reduced, barely extending beyond the posterior margin of coxae IV; peritreme absent. **Jacobsonia** s.str. Berl.
— Genital shield well developed, extending almost to the anal shield; peritreme present but reduced and extending only to the posterior margin of coxae III. **Jacobsonia** (**Parajacobsonia**) Evans
7. Claws on all legs relatively well developed; peritreme normal, extending beyond coxae I; chelicerae of males chelate-dentate. 8
— Claws on all legs vestigial (or absent?); dorsal shield not completely covering the dorsum in females; peritreme reaching beyond middle of coxae III; both digits of chelicerae of males reduced to slender processes without teeth; spermatophoral process long. **Hypoaspis** (**Julolaelaps**) Berl.
8. A pair of long, whip-like setae situated posteriorly on the dorsal shield. **Hypoaspis** (**Coleolaelaps**) Berl.
— Dorsal shield without these long whip-like setae on the dorsum. **Hypoaspis** (**Hypoaspis**) Can.

Genus **Hypoaspis** Canestrini 1885 s.lat.

The characters of the genus were discussed by Evans (1955). Species of three subgenera of *Hypoaspis*, namely *Hypoaspis* Can., *Coleolaelaps* Berl. and *Julolaelaps* Berl. have been collected off Myriapoda.

Subgenus *Hypoaspis* Can.

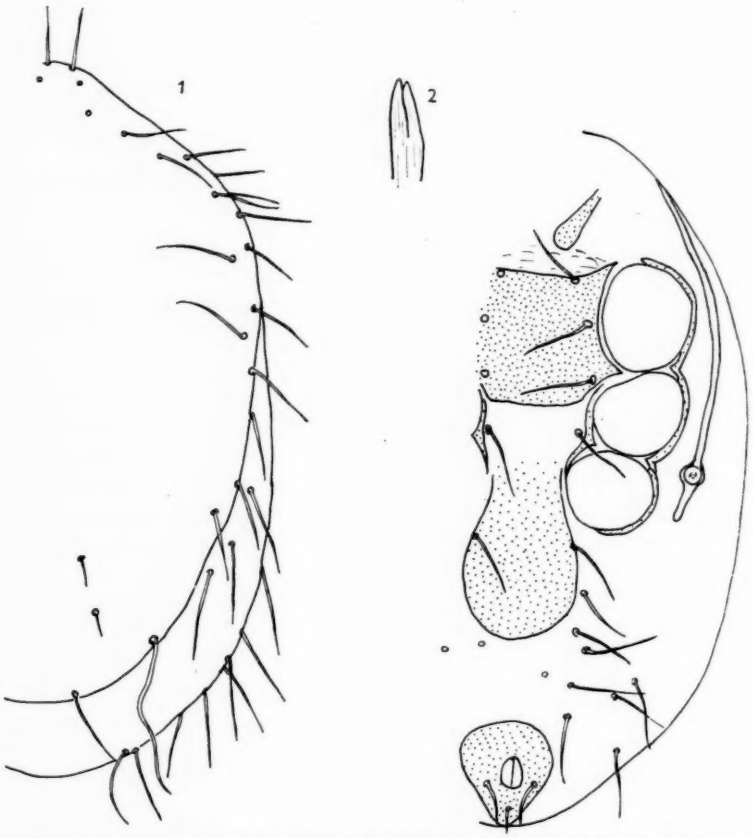
After a careful study of the descriptions and figures of *Hypoaspis* (*Hypoaspis*) *spirostrepti* Oudms, 1914, given by Oudemans (1915) and Vitzthum (1921) it is evident that this species belongs to the subgenus *Julolaelaps* and that *J. rotundatus* Berl. is probably a synonym of it. The species *Hypoaspis* (*Hypoaspis*) *indicus* Vitzthum, 1921, agrees with the description given by Berlese (1916) of *Julolaelaps luctator* and is synonymous with the latter. The only true species of the subgenus *Hypoaspis* found associated with Myriapoda is *Hypoaspis* (*Hypoaspis*) *polydesmoides* Evans, 1955. In this species the peritreme reaches anterior to coxa I, the claws on all legs are relatively well developed and the digits of the male chelicerae are chelate-dentate.

Subgenus *Coleolaelaps* Berl.

The subgenus *Coleolaelaps* is also represented by only one species, *Hypoaspis* (*Coleolaelaps*) *amazon* Berl., 1920. The type specimen was figured by Dr G. O. Evans in Florence (Berlese collection) and the figures are reproduced here (Figs. 1–3). Apart from the pair of long, whip-like setae (Fig. 1) this species is also characterized by strong spines on the legs with long setae on the metatarsus. The tips of the corniculi are bifurcate (Fig. 2). The venter of this species (Fig. 3) is typically hypoaspid. The sternal shield bears the usual three pairs of setae; the metasternal setae are situated on the integument; the genital shield is flask-shaped and provided with one pair of setae; the para-anal setae are situated in a line with the posterior margin of the anal opening; the peritreme reaches anteriorly beyond coxa I; the peritrematal plate is produced behind the stigma; all the setae on the venter are relatively long.

Subgenus *Julolaelaps* Berl. (type: *Julolaelaps dispar* Berl., 1916)

The 'genus' *Julolaelaps* was defined by Berlese as having the characters of *Hypoaspis* but the body is robust, more heavily sclerotized and the claws are absent on all the legs. Careful examination of the ambulacra, however, shows that in most (if not all) species small claws are present. These are probably functionless. It is also interesting to note that in the species studied by the present author the



Figs. 1–3. *Hypoaspis* (*Coleolaelaps*) *amazon* Berl., female. Fig. 1. Dorsum. Fig. 2. Tip of corniculus. Fig. 3. Venter.

claws or claw remnants are relatively most prominent on leg I and the least prominent on leg IV. The fact that the claws seldom protrude from the pulvilli, together with the fact that the specimens of Berlese were probably mounted in balsam, may well be the reason why they were overlooked.

The dorsal shield does not completely cover the dorsum of the females and is surrounded by a pseudomarginal shield. The peritreme is usually shorter than those in other subgenera of *Hypoaspis*. The males can readily be recognized by the

absence of chelate-dentate chelicerae; the digits are reduced to slender processes without teeth and the movable digit bears a long spermatophoral process. The males probably do not feed. The venter may be covered by a sterniti-genital shield and an anal shield or, as in the majority of species, by a holoventral shield. The dorsum may be partly or entirely covered by the dorsal shield. The specialized seta on the pedipalp of both sexes is two-pronged.

Key to the species of Julolaelaps (*females*)

1. Breadth of genital shield considerably less than that of anal shield; majority of the dorsal setae minute. 2
— Breadth of genital shield equal to or greater than that of anal shield. 4
2. Diameter of metapodal shields approximately the same as breadth of genital shield. **H. (Julolaelaps) luctator** Berl.
— Diameter of metapodal shields considerably less than breadth of genital shield. 3
3. Peritreme extending to anterior margin of coxa II. **H. (Julolaelaps) myriapodalis** sp.nov.
— Peritreme extending to anterior margin of coxa III. **H. (Julolaelaps) peritremalis** sp.nov.
4. Anal shield considerably longer than broad; para-anal setae situated in a line with anterior margin of anal opening. **H. (Julolaelaps) dispar** Berl.
— Anal shield slightly longer than broad; para-anal setae in line with posterior margin of anus. 5
5. Three setae on anal shield approximately of equal length; dorsal shield with 30 pairs of setae. **H. (Julolaelaps) spirostrepti** (Oudms)
— Post-anal seta relatively well developed, para-anals small; dorsal shield with 40 pairs of setae. **H. (Julolaelaps) pararotundatus** sp.nov.

Hypoaspis (Julolaelaps) peritremalis sp.nov.

Female

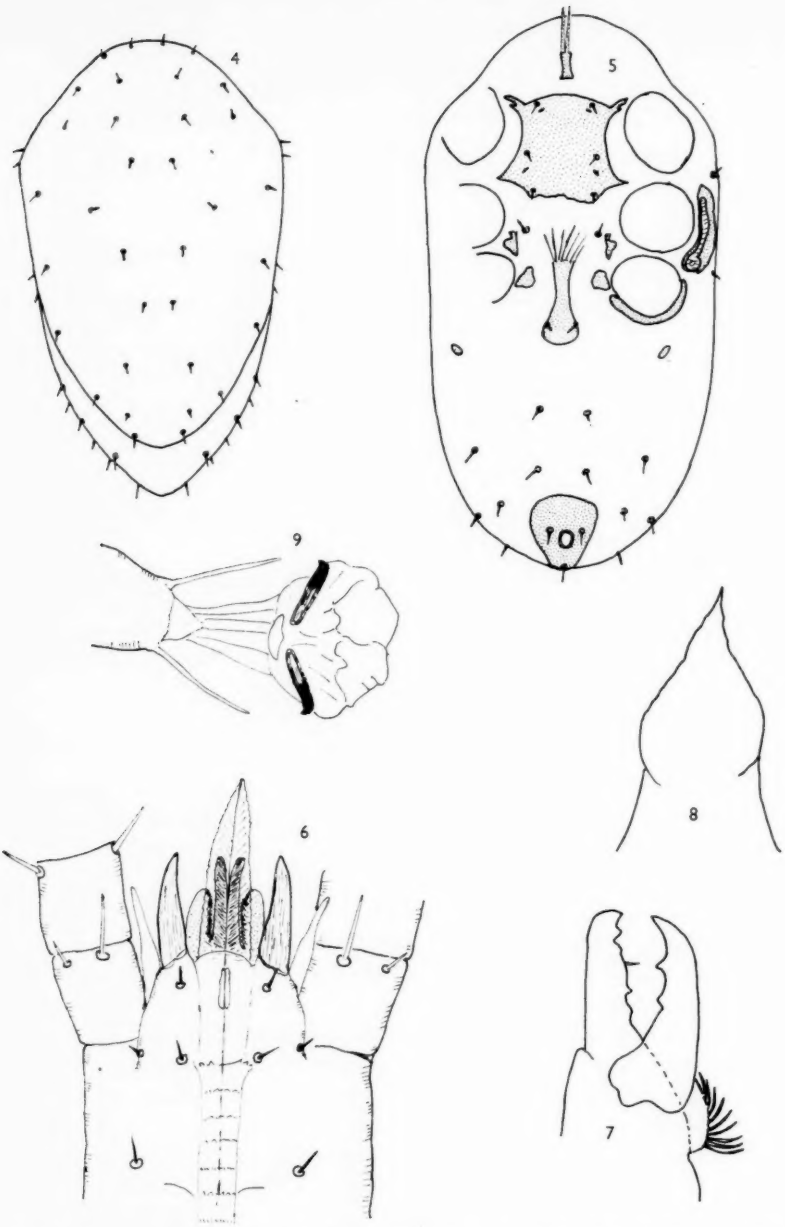
The idiosoma of the older female is strongly sclerotized, whereas the sclerotization of the young female resembles the nymphs. The young females can also easily be recognized by their smaller size.

Dimensions. Adult female: length, 660μ; breadth, 396μ. Young female: length, 594μ; breadth, 320μ.

It is, however, striking that the shields on the dorsum and venter do not increase in size to the same extent as the softer parts of the body. The shape of the body of the young females, tapering posteriorly, is similar to that of the males.

Dorsum. The dorsal shield (length, 550μ) bears 17 pairs of short simple setae (Fig. 4). Unlike other species of the genus, the vertical setae are short. In the adult females the dorsal shield is surrounded by a sclerotized pseudomarginal shield bearing simple setae as shown in Fig. 4.

Venter (Fig. 5). The sternal shield has a slightly convex anterior margin. The usual three pairs of setae and two pairs of pores are present. The genital shield (breadth, 44μ), bearing a pair of setae, is small and resembles the condition in *Julolaelaps luctator* Berl. and *J. myriapodalis* sp.nov. The metasternal setae are situated on the integument; metasternal shields are absent. The metapodal shields are small and oval-shaped. The interscutal membrane on the venter (excluding the lateral sides) is provided with six pairs of setae. The anal shield (length, 88μ;



Figs. 4–9. *Hypoaspis* (*Julolaelaps*) *peritremalis* sp.nov., female. Fig. 4. Dorsum. Fig. 5. Venter. Fig. 6. Gnathosoma. Fig. 7. Chelicera. Fig. 8. Tectum. Fig. 9. Ambulacrum, leg I.

breadth, 77 μ) has a more or less triangular shape. The para-anal setae are situated in a line with the anterior margin of the anal opening. The tritosternum has two finely ciliated laciniae which are almost twice the length of the base. The peritreme and peritrematal shield are reduced in comparison with the other species of *Julolaelaps*. The stigma is situated in a line between coxae III and IV and the peritreme reaches anteriorly to the front margin of coxa III.

Gnathosoma (Fig. 6). The usual four pairs of gnathosomal setae are present, of which the external posterior rostrals are the shortest. Seven rows of teeth can be distinguished in the ventral groove. The corniculi extend beyond the middle of the palp femur. The ligula is prominent and reaches far anteriorly. The chaetotaxy of the palp trochanter, femur and genu is normal (2–5–6). The specialized seta on the palp tarsus is two-pronged. The chelicerae (Fig. 7) are dentate, the fixed digit bearing three to four teeth and a small pilus dentilis. The movable digit is bidentate. The tectum (Fig. 8) is hyaline and is produced into a point anteriorly. The edges of the tectum are not smooth.

Legs. All the legs terminate in a pulvillus with vestigial claws. Those on leg I (Fig. 9) are more strongly developed than the others. It is sometimes necessary to orientate the ambulacra to be able to see the claws—especially the very small claws on leg IV. It is obvious that the pulvilli are used to adhere to the host animal and that the claws are vestigial. The setae on the legs are simple, those on tarsus I being the longest. The anterior dorsal part of femur III and IV each bear a strongly developed seta.

Male

Dimensions. Length, 583 μ; breadth, 385 μ.

The shape of the male is approximately the same as that of a young female. The venter is illustrated in Fig. 10. It differs from other known males of the subgenus in not having a holoventral shield. The sterniti-genital shield bears five pairs of setae. The anal shield resembles that of the female. The distribution of the setae on the venter is illustrated in the figure. The chaetotaxy of the dorsum is essentially the same as that of the young females.

The gnathosoma is the same as that of the female except for the chelicerae. The 'digits' are without teeth and the 'movable digit' bears a large spermatophoral process (Fig. 11). The legs of the male differ from those of the female in the stronger development of the setae, many of which are spine-like (Fig. 10). The most conspicuous spines are two on the ventral side of femur IV.

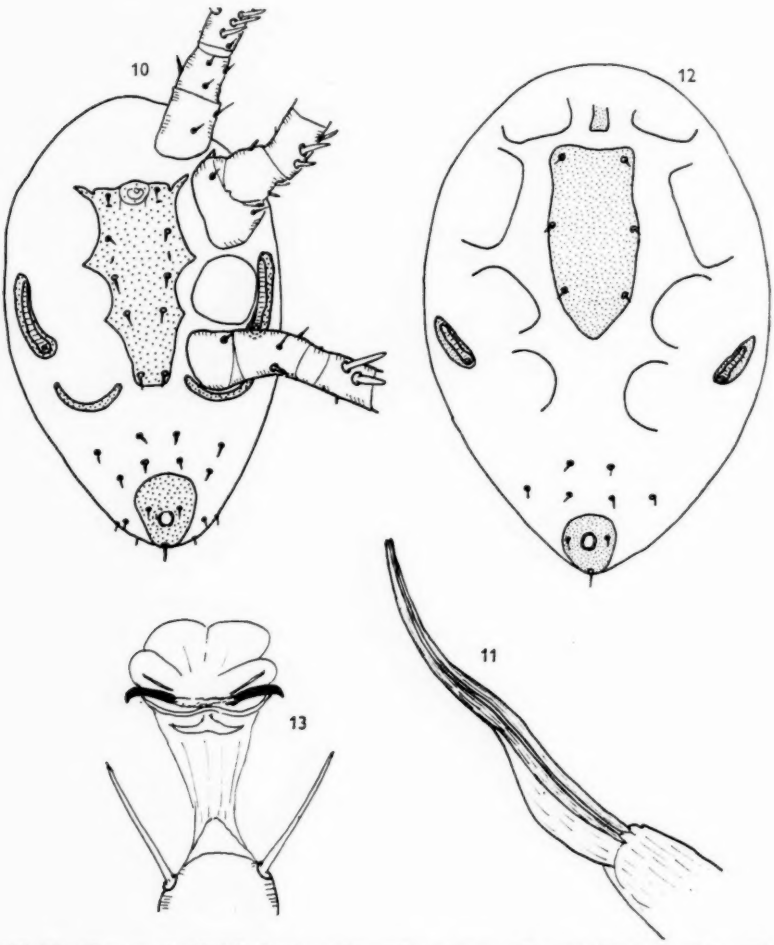
Protonymph (Fig. 12)

Dimensions. Length, 440 μ; breadth, 275 μ.

A striking feature of the venter of the protonymph is the presence of only three pairs of setae in the intercoxal region. The distribution of the setae is shown in the figure. The dorsal chaetotaxy is essentially the same as that of the adults. The dorsal shield does not cover the entire dorsum. The peritrematal shield and the peritreme are relatively shorter than those of the adults and the claws on the legs

are more prominent (Fig. 13). The chaetotaxy on the trochanter, femur and genu of the palp is 1–4–5 (see Evans, 1957, p. 215).

Host and locality. Four females, two males and one protonymph off a spirostreptid, West Africa. Collected by E. A. J. Duffy.



Figs. 10–13. *Hypoaspis* (*Julolaelaps*) *peritremalis* sp.nov. Fig. 10. Venter, male. Fig. 11. Spermatophoral process on movable digit of chelicera, male. Fig. 12. Venter, protonymph. Fig. 13. Ambulacrum, leg I, protonymph.

Hypoaspis (Julolaelaps) myriapodalis sp.nov.

This species is well sclerotized in the adult stage. As in the previous species the older adults have a larger and more heavily sclerotized body than the young adults. The shields, however, are of the same dimensions.

Female

Dimensions. Old adult: length, 924μ; breadth, 583μ. Young adult: length, 737μ; breadth, 440μ.

The venter (Fig. 14) closely resembles that of *J. peritremalis* and *J. luctator*. It can, however, easily be distinguished from the former species by the longer peritreme which reaches anteriorly to the front margin of coxa II. The new species can be separated from *J. luctator* by the presence of small metapodal shields.

The posterior margin of the sternal shield is concave. The genital shield (breadth, 66μ) is long and narrow as in the above-mentioned species. The triangular-shaped anal shield (length, 110μ; breadth, 100μ) has a strongly sclerotized lateral and anterior margin. The para-anal setae are situated in a line with the anterior margin of the anus. The chaetotaxy of the venter is illustrated in the figure. The tritosternum is normal.

Dorsum (Fig. 15). The dorsal shield (length, 715μ) bears twenty pairs of setae, the majority of which are minute. The vertical setae are relatively long and conspicuous. Four pairs of setae on the margin of the shield have large setal bases. These setae are longer than the others (excluding the verticals). The pseudo-marginal shield is well sclerotized in the 'old' female. The distribution of the setae is shown in the figure.

Gnathosoma (Fig. 16). The capitular setae are the longest of the four pairs of gnathosomal setae. The ventral groove is provided with six rows of teeth. Both digits of the chelicerae (Fig. 17) are bidentate. The fixed digit bears a pilus dentilis. The hyaline tectum (Fig. 18) has a median pointed mucro. The margin is incised so that the tectum is divided into small lobules.

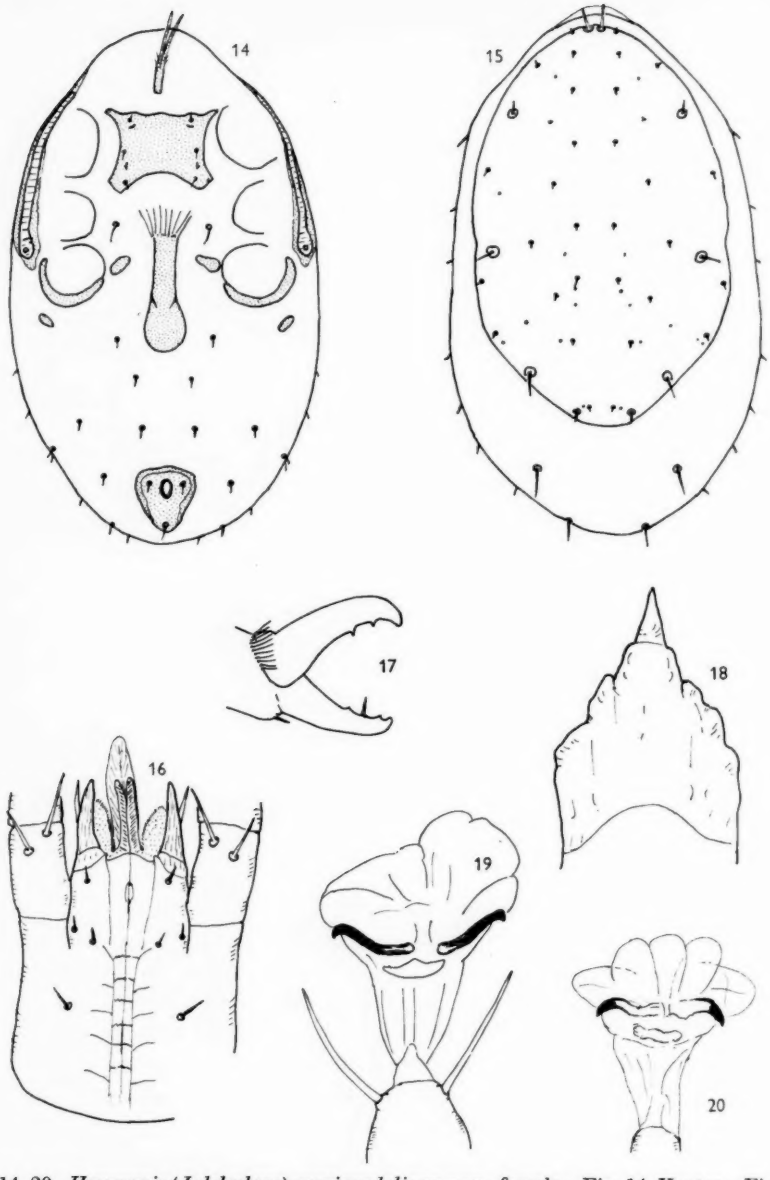
Legs. The legs each bear a pulvillus with small claws, those on leg I (Fig. 19) being the stronger. The claws on the other legs (Fig. 20) are small. All the claws, however, are vestigial. The setae are simple except for a spine-like seta on each of femurs II–IV. A few other setae are strongly developed but are not actually spinose.

Male

Dimensions. Length, 759μ; breadth, 495μ.

The chaetotaxy of the dorsum is essentially the same as that of the female. The dorsal plate covers the entire dorsum. A striking difference, however, is that the submarginal setae are not as short as in the female but are about the length of the vertical setae. The latter are relatively longer than those of the female. The setae on the ventral interscutal membrane are longer. The male has a holoventral shield (Fig. 21). The shield is provided with eight pairs of simple setae in addition to the three anal setae. Other features of the venter are as in the female.

The general structure of the gnathosoma is the same as that of the female. The digits of the chelicerae are reduced to slender processes (Fig. 22) without teeth. The spermatophoral process is long and incurved. The legs are provided with stronger setae than those of the female. Some of these tend to be spine-like (Fig. 23). Leg IV (Fig. 24) bears a few relatively long setae on its tarsus. In the female the corresponding setae are short.

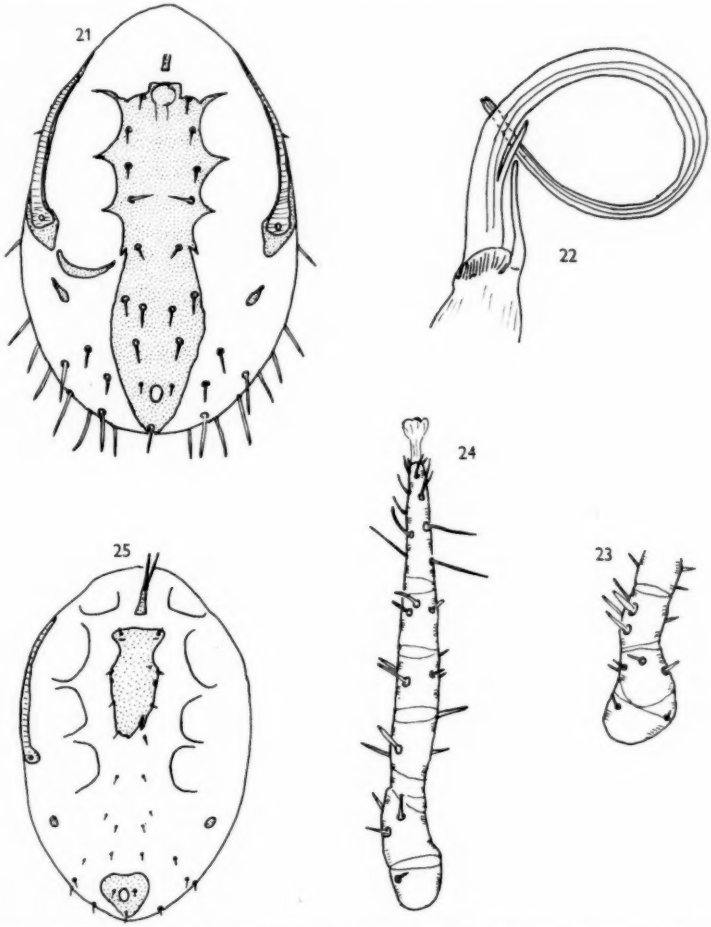


Figs. 14–20. *Hypoaspis* (*Julolaelaps*) *myriapodalis* sp.nov., female. Fig. 14. Venter. Fig. 15. Dorsum. Fig. 16. Gnathosoma. Fig. 17. Chelicera. Fig. 18. Tectum. Fig. 19. Ambulacrum, leg I. Fig. 20. Ambulacrum, leg III.

Deutonymph (Fig. 25)

Dimensions. Length, 759μ; breadth, 495μ.

These are the measurements of the largest of the two deutonymphs. The intercoxal region is provided with ten setae of which seven are situated on the 'sternal' shield. In both specimens one seta IV is off the shield. The distribution of the



Figs. 21–25. *Hypoaspis* (*Julolaelaps*) *myriapodalis* sp.nov. Fig. 21. Venter, male. Fig. 22. Chelicera, male. Fig. 23. Leg II, male. Fig. 24. Leg IV, male. Fig. 25. Venter, deutonymph.

setae on the venter is illustrated in the figure. The vertical setae are short, but the marginal setae on the dorsum are long. All the legs are provided with small claws developed to about the same degree as those of the adults. The chaetotaxy of the palp trochanter, femur and genu is the same as that of the adults.

Host and locality. Off spirostreptid among bananas imported from West Africa; seven females, three males and two deutonymphs.

Hypoaspis (Julolaelaps) pararotundatus sp.nov.

This is the largest of the three new species and can readily be separated from the other two by the presence of a large genital shield. The old adults are very well sclerotized.

Female

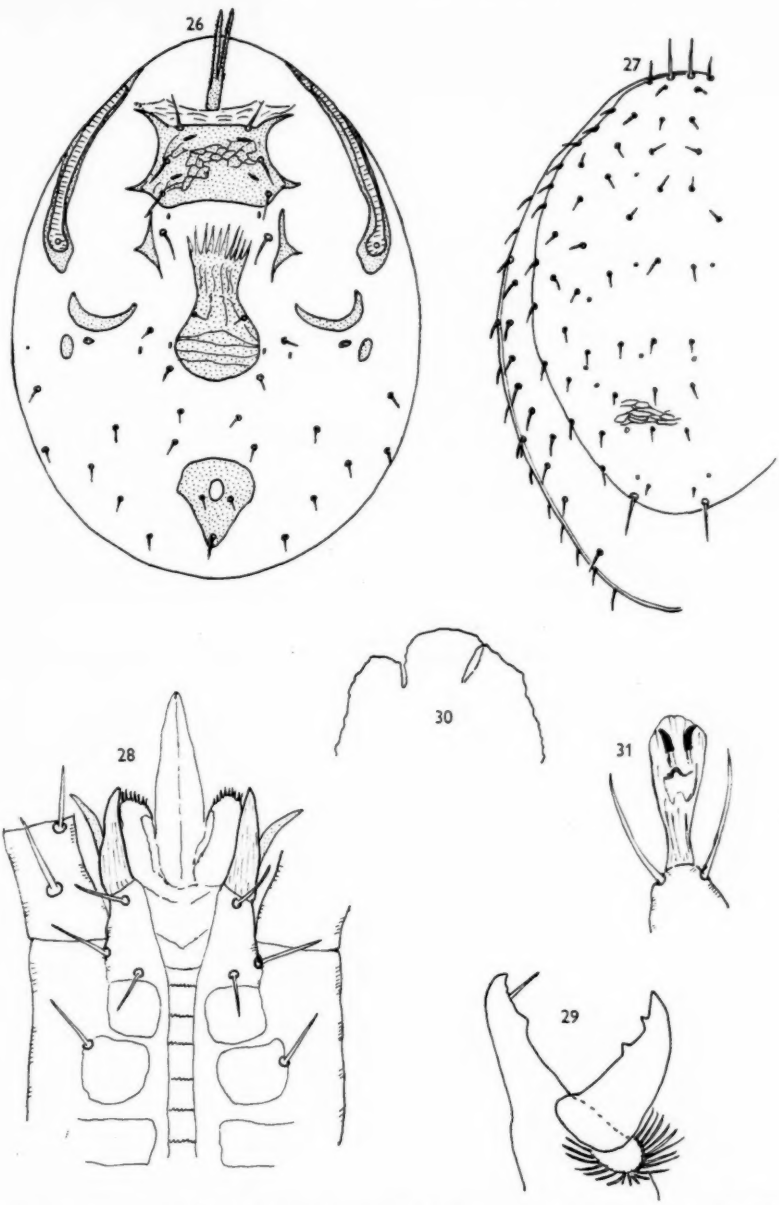
Dimensions. Old adult: length, 1177μ; breadth, 770μ. Young adult: length, 1056μ; breadth, 627μ.

Venter (Fig. 26). The venter shows a marked resemblance to *J. spirostrepti* (Oudms). The reticulated sternal shield is broader than long and bears three pairs of setae and two pairs of conspicuous pores. The metasternal setae are situated on the integument. The membrane anterior to the sternal shield is sclerotized but does not form a pre-endopodal plate as in *J. spirostrepti*. The genital shield is broader (breadth, 170μ) than the anal shield (breadth, 150μ). The latter tapers more posteriorly than in *J. spirostrepti*. The para-anal setae are situated in a line with the posterior margin of the anus. The metapodal plates are represented by a larger and a smaller one on each side. The genital shield is flanked by two pairs of setae and two pairs of minute platelets. The anterior pair of setae are absent in the drawing of Oudemans. The distribution of the setae on the venter is illustrated in the figure. The stigma is situated between coxae III and IV and the peritrematal shield is produced slightly behind the former. Anteriorly the peritreme extends to the anterior margin of coxa II. The two laciniae of the tritosternum are finely setose.

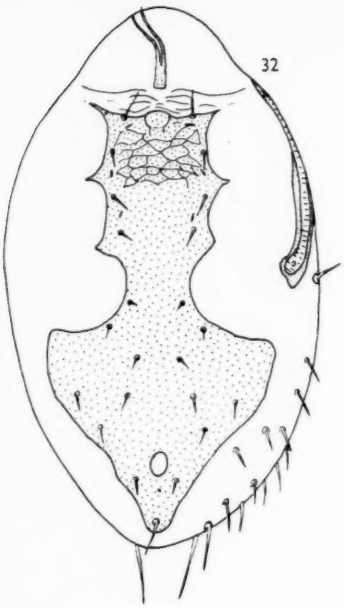
Dorsum (Fig. 27). The reticulated dorsal shield (length 1012μ) bears 40 pairs of setae, whereas Oudemans gives 30 pairs for *J. spirostrepti*. Most of the setae are short and simple. Exceptions are the long verticals and the posterior pair on the shield. The pseudomarginal shield is very well sclerotized in the old adult and bears eleven pairs of setae. The strip of transparent interscutal membrane bordering it laterally is provided with 12–13 pairs of simple setae. The chaetotaxy of the dorsum and the distribution of the pores are illustrated in the figure.

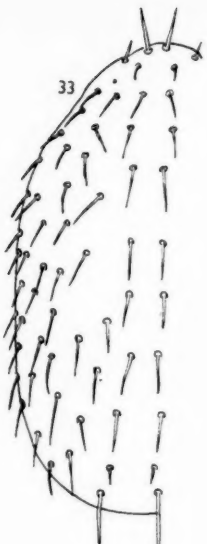
Gnathosoma (Fig. 28). Very similar to that of other species described. The gnathosomal setae are relatively well developed and of approximate equal length. The ventral groove has six rows of teeth. The ligula is as prominent as in the other species. The movable digit of the chelicera (Fig. 29) is bidentate and the fixed digit has one large tooth and a few minute ones at the tip. The pilus dentilis is prominent. The specialized seta on the palp tarsus is two-pronged. Distally the tarsus is provided with a slender sickle-shaped seta. The tectum (Fig. 30) is hyaline as in the other species and shows three lobes anteriorly as in *J. spirostrepti*.

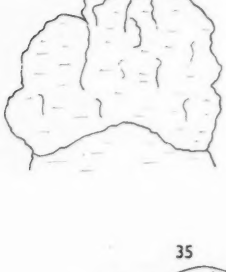
Legs. The legs are provided with pulvilli and small claws which are better developed on leg I (Fig. 31). The antero-dorsal surface of femurs II–IV each bear a strongly developed seta, the one on femur IV being the strongest.

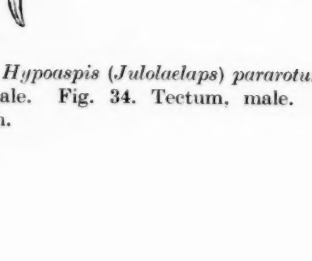


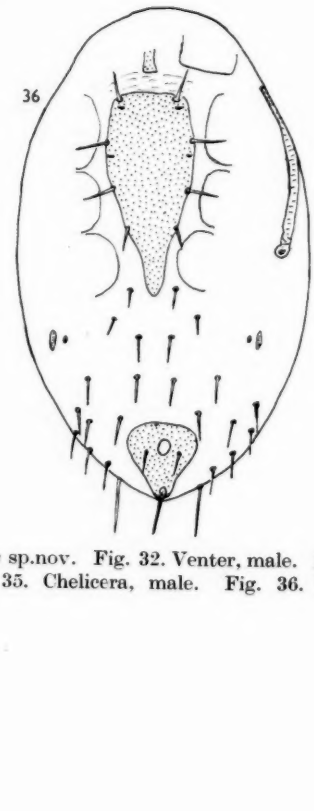
Figs. 26–31. *Hypoaspis* (*Julolaelaps*) *pararotundatus* sp.nov., female. Fig. 26. Venter. Fig. 27. Dorsum. Fig. 28. Gnathosoma. Fig. 29. Chelicera. Fig. 30. Tectum. Fig. 31. Ambulacrum leg I.











Figs. 32–6. *Hypoaspis* (*Julolaelaps*) *pararotundatus* sp.nov. Fig. 32. Venter, male. Fig. 33. Dorsum, male. Fig. 34. Tectum, male. Fig. 35. Chelicera, male. Fig. 36. Venter, deutonymph.

Male

Dimensions. Length, 880μ; breadth, 484μ.

The holoventral shield (Fig. 32) is reticulated and bears ten pairs of setae in addition to the three anal setae. The shield is broad behind coxae IV and then gradually tapers posteriorly. As in the female the region between the anterior margin of the shield and the base of the sternum is sclerotized. The chaetotaxy of the ventral interscutal membrane is shown in the figure. The reticulated dorsal shield covers the entire dorsum. The setae are much longer than in the female. Their distribution is illustrated in Fig. 33. The tectum (Fig. 34) of the male is essentially the same as in the female, but the anterior lobes are not distinctive. The typical chelicerae of *Julolaelaps* are present (Fig. 35). The digits are reduced to toothless processes. The spermatophoral process is not as long as in *J. myriapodalis*. In addition to these structures the proximal part of the spermatophoral process is covered by a transparent leaf-like membranous structure. The legs are unarmed and similar to those of the female.

Deutonymph

Dimensions. Length, 847μ; breadth, 495μ.

The dorsum of the deutonymph resembles more closely that of the male than the female. The setae are even longer than those of the male. The dorsal shield is faintly reticulated and does not cover the entire dorsum. The chaetotactic pattern is the same as that of the female.

The venter (Fig. 36) is covered by a 'sternal' shield, bearing four pairs of setae and an anal shield with a prominent post-anal seta. The area in front of the sternal shield is slightly sclerotized. The metapodal shields are similar to those of the female. The distribution of the setae on the venter is shown in the figure. The gnathosoma and the chaetotaxy of the palp is normal. The tectum has three distinct lobes anteriorly. The claws on the legs are more prominent than in the adults.

Host and locality. Four females, seven males and one deutonymph off a spirostreptid, Pemba, West Africa.

Hypoaspis (Julolaelaps) dispar Berl., 1916

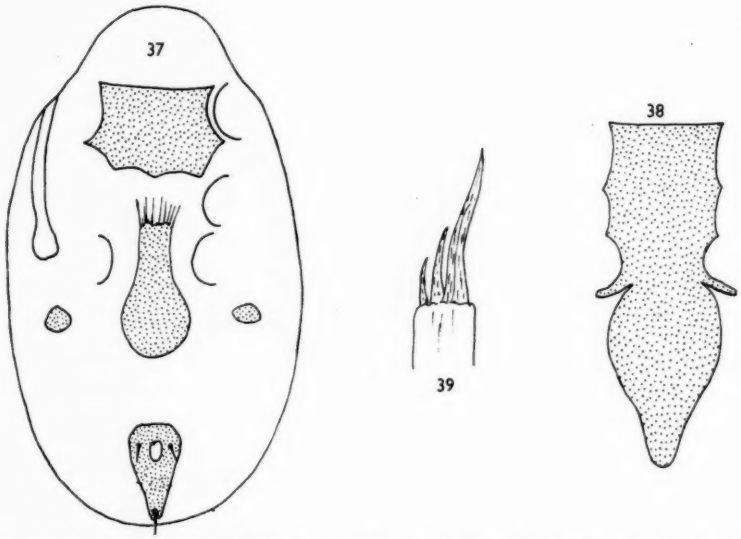
Berlese (1916) gave brief descriptions of the female and male but no figures. The only figures available are the diagrammatic sketches in Berlese's notebook. These are reproduced in Figs. 37–39. The drawing of the venter of the female (Fig. 37) shows the genital shield wider than the anal shield. The latter is characteristic in that it is long and narrow. The dimensions given by Berlese are: length, 1500μ; breadth, 1000μ. The male is unarmed and smaller than the female (1120 × 650μ). The venter is covered by a holoventral shield (Fig. 38). The chelicerae are typical of the subgenus as shown in Fig. 39.

Host and locality. On julid, Italian Somaliland (Goriei).

Hypoaspis (Julolaelaps) luctator Berl., 1916

Syn.: *Hypoaspis indicus* Vitzth., 1921.

This species was adequately described by Vitzthum (1921). Berlese's description agrees with the description given by Vitzthum for *Hypoaspis indicus* and is thus considered a synonym of the former. This species can be readily recognized by the large metapodal shields and the strong spines and spurs on the legs. Vitzthum also



Figs. 37–39. *Hypoaspis* (*Julolaelaps*) *dispar* Berl. (based on Berlese's notebook). Fig. 37. Venter, female. Fig. 38. Holoventral shield, male. Fig. 39. Chelicera, male.

described and figured the male. Unfortunately he was unable to describe the chelicerae but it can be assumed that this species has the characteristic male chelicerae with the long spermatophoral process. He stated the following: 'Die Mandibulae konnten nicht herauspräpariert werden. Sicher ist, dass sie sehr denen von *Hypoaspis Krameri* Canestrini ♂ ähneln, namentlich in Bezug auf den sehr langen, hakenförmigen Anhang am Digitus mobilis' (Vitzthum, 1921, p. 21). The author made no mention of the degree of development of the claws on the legs.

Hosts and localities. Berlese's specimen was found on a julid, Italian Somaliland (Goriei and El Ualae). Vitzthum's specimens were found on *Spirostreptus* sp. from India.

Hypoaspis (Julolaelaps) spirostrepti (Oudms) 1914

Syn.: *Julolaelaps rotundatus* Berl. 1916.

This species was described by Oudemans (1914). In 1915 he gave a more detailed description and figures of the female. Vitzthum (1921) described the male. Judging from a figure of a badly damaged specimen of *J. rotundatus* made by Dr G. O. Evans, this species is a synonym of *J. spirostrepti*. The venter of this

species resembles that of *J. pararotundatus* sp.nov. According to Oudemans the dorsal shield of the female is provided with 30 pairs of setae. Neither Oudemans nor Vitzthum mention the claws in their descriptions. The male has a holoventral shield and the shield covers the entire dorsum. As regards the male chelicerae, Vitzthum stated that he could not study the gnathosoma. It can be assumed that these structures are typical.

Hosts and localities. Berlese's specimen was found on a julid, Italian Somaliland (El Ualae). Oudemans found his on *Spirostreptus* sp., German East Africa. Vitzthum's specimen on *Spirostreptus* sp., India.

Genus **Scissuralaelaps** Womersley, 1945

Womersley (1945, p. 225) gave the following characters for the genus: '*Hypoaspis*-like. Lightly chitinized with moderately long to short fine dorsal setae. Female with sternal shield divided longitudinally, ventral shield fused with genital, widely separated from anal. Pre-endopodal shields present or absent. Metasternal shields only represented by seta and pore. Dorsal shield entire, not entirely covering body. Male with sternal, genito-ventral and [anal] shields coalesced. Legs without strong spines or processes. Mandible with process on movable finger of chelicerae.' Two species of this genus are known but only the type species was found on millipedes.

Scissuralaelaps nova-guinea Wom., 1945

Pre-endopodal shields present. Sternal shield completely divided longitudinally in female. Chelicerae in male dentate. Peritreme relatively long.

Host and locality. Off a millipede on orchids, New Guinea.

Genus **Iphiolaelaps** Womersley, 1956

The characters of the genus given by Womersley (1956, p. 541) are the following: 'Iphiopsidae, with all tarsi with carucles but without claws. Female pre-endopodal shields of coxae I, II, and III fused on each side to form the separated halves of the longitudinally divided sternal shield, each half furnished with three setae and the corresponding pores; metasternal shields only represented by the setae; genital shield, small, drop-like and fringed anteriorly, with one pair of setae; ventral shield wanting; anal small with three setae; a small metapodal shield on each side, behind coxae IV. Male: sterno-genital shield with five pairs of setae, posterior end irregularly rounded on level with posterior margin of coxae IV, behind with four transverse lenticular scutelets. Chelicerae with movable finger much longer than fixed finger and with even longer spermatophore carrier. Legs unarmed.'

Iphiolaelaps myriapoda Wom., 1956.

The type and only known species of the genus. The generic characters were based on this species. The presence of a stigma without peritreme can be added to the list.

Host and locality. From millipedes, Mt. Lamington, Queensland, Australia.

List of laelaptid paraphages of Myriapoda

Jacobsonia (*Jacobsonia*) *submollis* Berl., 1910
Jacobsonia (*Jacobsonia*) *minor* Berl., 1910
Jacobsonia (*Jacobsonia*) *audyi* Evans, 1955
Jacobsonia (*Parajacobsonia*) *tertia* Vitzth., 1931
Scolopendracarus brevipilis Evans, 1955
Iphiopsis miriabilis Berl., 1882.
Scissuralaelaps nova-guinea Wom., 1945
Iphiolaelaps myriapoda Wom., 1956
Hypoaspis (*Hypoaspis*) *polydesmoides* Evans, 1955
Hypoaspis (*Coleolaelaps*) *amazon* Berl., 1920
Hypoaspis (*Julolaelaps*) *dispar* Berl., 1916
Hypoaspis (*Julolaelaps*) *luctator* Berl., 1916
Hypoaspis (*Julolaelaps*) *spirostrepti* (Oudms), 1914
Hypoaspis (*Julolaelaps*) *peritremalis* sp.nov.
Hypoaspis (*Julolaelaps*) *myriapodalis* sp.nov.
Hypoaspis (*Julolaelaps*) *pararotundatus* sp.nov.

SUMMARY

1. A key to the laelaptid paraphages of Myriapoda is given.

2. *Hypoaspis* (*Hypoaspis*) *spirostrepti* Oudms is transferred to the subgenus *Jululaelaps*. *J. rotundatus* Berl. is regarded as a synonym of this species.

3. *Hypoaspis* (*Hypoaspis*) *indicus* Vitzth. is regarded as a synonym of *Julolaelaps luctator* Berl.

4. *Hypoaspis* (*Coleolaelaps*) *amazon* is figured.

5. The subgenus *Julolaelaps* is defined and a key given to the species. The type species is figured.

6. Three new species are described, namely *Hypoaspis* (*Julolaelaps*) *peritremalis*, *H.* (*J.*) *myriapodalis* and *H.* (*J.*) *pararotundatus*.

7. A list of all the known species of Laelaptidae occurring on millipedes is given.

REFERENCES

BERLESE, A. (1916). Centuria prima di Acari nuovi. *Redia*, **12**, 19–67.
BERLESE, A. (1920). Centuria quinta di Acari nuovi. *Redia*, **14**, 143–95.
EVANS, G. O. (1955). A review of the laelaptid paraphages of the Myriapoda with descriptions of three new species (Acarina: Laelaptidae). *Parasitology*, **45**, 352–68.
EVANS, G. O. (1957). An introduction to the British Mesostigmata (Acarina) with keys to families and genera. *J. Linn. Soc. Lond.* **43**, 203–59.
OUDEMANS, A. C. (1914). Acarologische aanteekeningen. 52. *Ent. Ber.* **4**, 65–73.
OUDEMANS, A. C. (1915). Notizen über Acari. 22. *Archiv Naturgesch.* **81**, Abt. A, Hft 1, 122–80.
VITZTHUM, H. (1921). Acarologische Beobachtungen. 4. Reihe. *Archiv Naturgesch.* **86**, Abt. A, Hft 10, 1–69.
WOMERSLEY, H. (1945). An interesting and primitive new genus of Laelaptidae (Acarina) from Australia and New Guinea. *Rec. S. Aust. Mus.* **8**, 225–8.
WOMERSLEY, H. (1956). On some Acarina—Mesostigmata from Australia, New Zealand and New Guinea. *J. Linn. Soc.* (*Zool.*), **42**, 505–99.

(*MS. received for publication* 10. XI. 1957.—Ed.)

STUDIES ON PARASITIC WORMS OF SHEEP IN SCOTLAND

I. EMBRYONIC AND LARVAL DEVELOPMENT OF *HAEMONCHUS CONTORTUS* AT CONSTANT CONDITIONS

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(With 2 Figures in the Text)

Most of the work on the biology of the sheep stomach worm, *Haemonchus contortus*, has been done in South Africa, the Southern United States and Australia, under climatic conditions different from those which obtain in Britain. Nevertheless, these findings have been applied in this country, in the design of rotational grazing systems, to avoid outbreaks of parasitic gastro-enteritis.

A priori, it might be expected that the temperature relations of development, and the developmental thresholds of nematodes are not the same in Britain as in subtropical or warm-temperate regions. It was desirable, therefore, to determine how far existing information was applicable to British conditions.

The present paper is concerned with some laboratory studies on the factors affecting the free-living stages of *H. contortus* and includes the results of some parallel observations on the eggs and larvae of *Ostertagia* spp., *Trichostrongylus* spp., and *Oesophagostomum venulosum*.

Ransom (1906), working in the U.S.A., reported that some eggs of *H. contortus* in cultures of faeces, when kept at a temperature of 16–20° C., hatched within 2 days, while others in the same culture did not hatch until a week had elapsed. He found that eggs showed little resistance to the adverse effects of drying or freezing, although in 1907 he stated that: 'If the temperature is above 40–50° F. [4·4–10° C.] the eggs hatch out requiring from a few hours to 2 weeks, according as the temperature is high or low. When the temperature is below 40° F. the eggs remain dormant, and in this condition may retain their viability for 2 or 3 months, afterwards hatching out if the weather becomes warmer.'

Ransom also reported that infective larvae appear in a faecal culture 'in three to four days... *if the temperature remains constant at about* 95° F. [35° C.]'.† At 21° C., 6–14 days are required and at an average of 10° C. (fluctuating between 7·8° and 14° C.), 3–4 weeks are necessary for the eggs to hatch and the larvae to develop to the infective stage.

Veglia (1915), in South Africa, prepared cultures of *H. contortus* eggs by comminuting, washing and sieving infected faeces and collecting the sediment for incubation. At temperatures ranging from 26° to 35° C. he found that the majority of eggs hatched within 14–24 hr., while at uncontrolled temperatures between 15° and 18° C. embryonation and hatching took place in about 50 % of the eggs after 4 days.

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† Our italics.

Veglia identified 40° C. as the upper temperature limit of larval development. At 37° C. development was rapid but accompanied by a very high mortality. He considered 35° C. to be within the 'normal' temperature range for larval development: migration of third-stage larvae up the side of the culture jar began on the fourth day. Between 35° and 22° C. which Veglia regarded as 'well-defined limits' larvae 'can grow without showing any marked variations'. The few observations made below 22° C. suggested that development required 6 days at 18° C., 8 days at 15° C. and 18–21 days at 8° C.

Clunies Ross & Gordon (1936), quoting unpublished observations by Kauzal in Australia, reported that *H. contortus* 'eggs develop best at temperatures of 25–30° C. (75–85° F.), and that development is slowed progressively by lower temperatures, and checked altogether at continuous temperatures of 5° C. (41° F.), at which the eggs die after periods of from 4 to 6 days'. At 25–30° C. the infective larval stage is reached in four to five days.

Shorb (1943, 1944), in the U.S.A., reported that no development took place in cultures kept at temperatures below 13° C., although a few eggs 'survived' at these temperatures for as long as 4 weeks. Shorb prepared his cultures of *H. contortus* eggs by 'pulverizing' faecal pellets and thoroughly mixing the resultant mass.

Dinaburg (1944), apparently working with the same strain, made observations on the rate of development and survival of cultures of *H. contortus* which were kept out of doors. He prepared his material by 'finely' grinding faecal pellets in a mortar before placing it on experimental grass plots. Unhatched viable eggs were found (by a salt-flotation technique) in cultures which had been exposed to temperatures above 21° C. for 6 days. In cultures exposed to temperatures below 21° C. some eggs remained viable after 13 days' exposure. He found, however, that such eggs 'do not hatch after exposure to mean maximum temperatures below 70° F. [21° C.] for more than 6 days'.

Dinaburg also concluded that no development of eggs or larvae took place at temperatures constantly below 65° F. Nevertheless, he presents data which show that some eggs and larvae developed at mean maximum air temperatures of 42°, 47°, 60° and 64° F. The so-called 'Dinaburg Line' (Kates, 1950), namely, a mean maximum temperature of 65° F. below which it is held that development cannot be completed, has been widely accepted. (According to Dinaburg's own statement, however (see below), the ecological limit is evidently even higher, namely a mean maximum of 70° F.) Gordon (1948), while holding this view to be applicable to Australian conditions, presents evidence which contradicts it. In his fig. 6 he shows the spring rise of *H. contortus*, which he assumes to be due to re-infestation, beginning in late August. A mean maximum temperature of 65° F. is not reached until the end of September.

MATERIAL AND METHODS

Faeces were taken from lambs maintained indoors with experimental monospecific infestations of *H. contortus*. The animals were on dry feed and their faeces were in pellet form. Egg counts, based on the McMaster method, ranged from 1000 to

38,000 per g. Culture was carried out in 1 lb. ($4\frac{1}{2}$ in. × 3 in. diam.) glass jam-jars covered with inverted Petri dishes to control loss of water through evaporation. The jars were partly filled (one-third to one-half), periodically moistened, and the pellets gently disturbed so that they were neither saturated nor mechanically broken. The water content was maintained at a level comparable to that of freshly passed faeces (60–70 % water) and is here termed 'normal moisture'.

Replicate experiments were set up dividing one lot of faeces into equal parts for comparison of development at different temperatures.

Observations were made on cultures kept at constant temperatures of 0·0°, 7·2°, 14·4°, 21·7° and 37° C. Culture samples were taken as frequently as conditions warranted. Samples were examined by direct microscopic observations on smear preparations. For each observation fifty individuals were classified according to developmental stage. The classification categories comprised four egg and three larval stages: E_1, early blastomere to morula; E_2, differentiating gastrula; E_3, tadpole or early vermiform; E_4, late vermiform to prehatch larva; L_1, first-stage larva; L_2, second-stage larva; L_3, third-stage larva.

In some cases, cultures at various stages of development were saturated with excess water, or dried, or transferred to low temperatures to determine the susceptibility to different adverse conditions. The criterion of survival adopted was resumption of development of the embryo after removal from the adverse condition to a more favourable one. Observations on samples taken to measure survival required 2–5 days' subsampling since the effects of cold or lack of oxygen seemed sometimes to cause a lag in the resumption of normal embryonation.

Studies on the rate of larval production and migration from pellets were carried out on both individual and groups of pellets. The technique involved is discussed in conjunction with the presentation of results in the next section.

Observations on *Ostertagia* spp., *Trichostrongylus* spp., and *Oesophagostomum columbianum* were made, using faeces from sheep with mixed infestations. Eggs were identified by means of Cunliffe & Crofton's (1953) technique. First- and second-stage larvae were found to be identifiable on the basis of predetermined measurements and by their resemblance to third-stage larvae. A similar technique has been developed by Whitlock (unpublished) for use at the McMaster Institute, Sydney. Whenever possible, identification of egg and larval stages in mixed infestations were confirmed by examination of the adult worms found in the intestine of the donor animal *post-mortem*.

RESULTS

Embryonation

Observations on the rate of embryonation and hatching of *H. contortus* showed that eggs in the same culture may behave in several ways: (1) eggs may develop at different rates; (2) eggs may exhibit intermittent states of active and inhibited development; and (3) eggs may fail to develop or die at any stage from unknown causes.

Because of these variable reactions and mortalities the data, on development

rates, have been presented as minimum and median times. The term median is, however, qualified here to have reference not to the total population, but to that part of it which completed development.

Temperature

The relation between temperature and embryonation rate, at conditions of normal moisture, is summarized in Table 1. Of the eggs cultured at 7·2° C. most became inhibited or failed to survive for prolonged periods; usually only 10%

Table 1. *The relation between various constant temperatures and the rate of embryonation, survival and hatching of* Haemonchus contortus

Temperature						
° C.	0·0	7·2	11·0	14·4	21·7	37
° F.	32	45	51	58	71	98
Time (days) required for development from blastomere stage						
Morula						
Minimum	—	3	<1	<0·5	<0·5	<0·25
Median	—	8	2	1	0·5	<0·5
Gastrula						
Minimum	—	6	<2	<1	<1	0·25
Median	—	18	3	2	1	0·5
Tadpole						
Minimum	—	14	3	1	<1	<0·5
Median	—	20	5	4	2	0·67
Prehatch						
Minimum	—	16	4	2	1	<0·5
Median	—	24	8	5	3	1
First-stage larvae						
Minimum	—	—	6	3	2	0·5
Median	—	—	10	6	4	1·5
Second-stage larvae						
Minimum	—	—	7	6	3	1
Median	—	—	13	10	5	2
Third-stage larvae						
Minimum	—	—	15	9	5	3
Median	—	—	20	12	8	4
Mean survival (%)						
Minimum	—	1	60	50	50	10
Maximum	—	10	90	80	70	30

developed and these required 16–24 days to reach the prehatch stage. Hatching seldom took place unless the eggs were transferred to a temperature above 9° C. The effect of temperature on the rate of embryonation is demonstrated in Fig. 1. These curves, unfortunately, are incomplete through insufficiency of intermediate points. There is evidence that the curves are sigmoidal in shape, the acceleration of development becoming proportionately smaller as temperatures increase above 30° C.

Mortality and inhibition vary with temperature. The proportion of eggs which survives to the prehatch stage varies between 10 and 90% with a maximum survival at 11° and 14·4° C. (Table 1). Of the eggs which fail to complete develop-

ment most die in the premorula or morula stage; mortality levels being much smaller in the gastrula and tadpole stages.

When eggs are allowed to develop to the prehatch stage before introducing them to low temperatures small proportions survive at $-2{\cdot}2$ to $1{\cdot}1°$ C. for as long as 2 months, although hatching does not take place until the temperature is raised to 9° C. Unembryonated eggs resist the adverse effects of $-2{\cdot}2$ to $1{\cdot}1°$ C. for only 3–7 days, after which time they succumb rapidly, all being dead by the end of 10 days.

Embryonated eggs (E_3 and E_4 stages) placed at $7{\cdot}2°$ C. survive for up to 4 months.

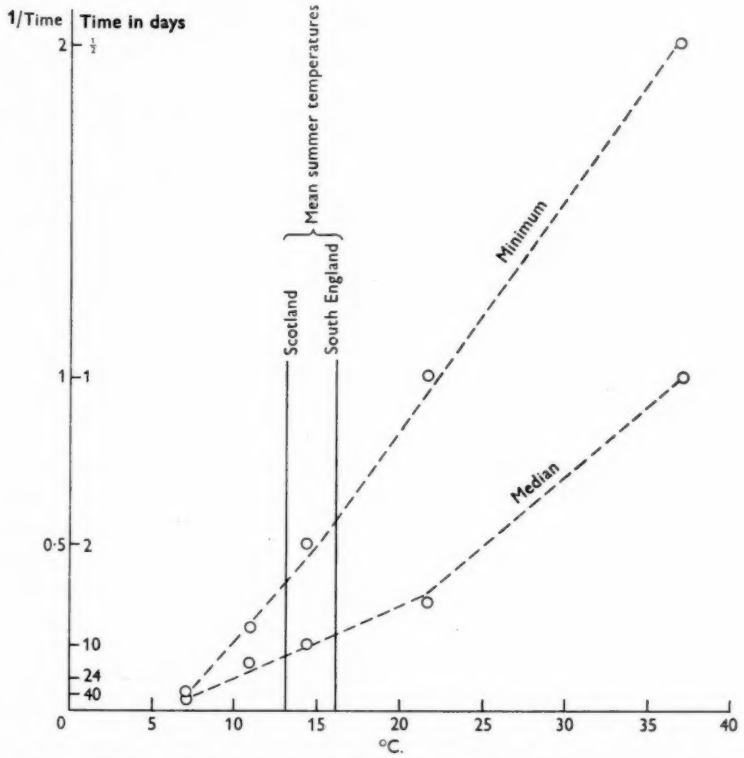

Fig. 1. The effect of temperature on the rate of embryonic development to prehatch stage of *Haemonchus contortus*.

Moisture and air

The effect of excess water on the developing embryo was investigated. A series of experiments was carried out to determine the rate of development of eggs in water and in saturated faecal masses. In one series, eggs which had been harvested from gravid females were put into 20 ml. of water in large test tubes (6 in. × 1 in.) that were partially immersed in a water bath maintained at 37° C. A slow continuous supply of air was bubbled through some of the egg suspensions. After

12 hr., samples were taken from the aerated and unaerated suspensions and the eggs examined microscopically. From the results shown in Table 2, it can be seen that embryonation was considerably inhibited in the unaerated suspension as compared with the aerated one.

Table 2. *Effects of aeration on the rate of embryonation of* Haemonchus contortus *eggs immersed under water for* 12 *hr. at* 37° C. *in the premorula stage*

	Number of individuals in different embryonic stages			
	Morula	Gastrula	Tadpole	Prehatch
Unaerated	350	130	40	1
Aerated	85	20	65	120

It will be noted that many of these eggs reached an advanced state of embryonation within 12 hr. of their removal from the parent worm. One would expect a comparable period of time to elapse between egg-laying by a female worm resident in the abomasum, and the dropping of the eggs in the faeces. Such eggs, however, do not embryonate beyond the morula stage in their passage down the gut. It appears probable that this phenomenon, to which Veglia (1915) has already referred, is due to an inhibition of development in the gut through oxygen inadequacy.

When faecal pellets are saturated with water, an increased proportion of their contained eggs becomes inhibited. This has been observed by other workers, including Shorb (1944) and Veglia (1915). This again appears to operate through interference with aeration.

Eggs were tested for their ability to survive when kept at different temperatures in faecal cultures which had been excessively moistened. Samples were collected at regular intervals from the saturated cultures, dried on filter-paper and kept slightly moist at 21·6° C. Smear preparations were examined at the time of sampling, and after 2–5 days, to see if further development took place after they were re-exposed to air (Table 3).

Table 3. *Survival of* Haemonchus contortus *eggs at different temperatures in saturated cultures of sheep pellets*

Temp. (° C.)	Days survived	
	Median	Maximum
21·7	26	33
14·4	40	56
11·0	45	81
7·2	21	68

Desiccation

Preliminary attempts to determine the effect of changes in the water content of sheep pellets on nematode eggs indicate that the relationships involved are complex, and much careful work needs to be done for a proper understanding.

Some preliminary results are reported here, since they are of general interest in assessing reactions of *H. contortus* eggs to the effects of drying.

The rate of water loss from pellets depends on the size of the pellet, and the temperature and relative humidity of the ambient air. The effect of drying on the eggs varies with the site within the pellets at which they are situated.

Sheep dung pellets vary considerably in size and consistency. For example, one of our lambs on an *ad lib.* hay diet produced pellets which ranged from 135 to 570 mg. on four different days within a 10-day period. Mean weights of random samples, of ten pellets each were as follows: $273 \pm 24{\cdot}1$, $238 \pm 9{\cdot}4$, $479 \pm 19{\cdot}0$, and $435 \pm 20{\cdot}1$ mg. Between animals, even greater variations occur: pellets of over 1 g. are not infrequent. An alteration in diet can cause marked changes in water content. The water content of pellets in the present work was in the range 55–70 %.

In atmospheres at or near saturation vapour pressure the water contents of pellets remains near the normal moisture level; in them, development proceeds uninterruptedly up to the third larval stage. When, however, drying takes place, the net effect on the worm population depends upon a combination of direct temperature effects and the evaporation rate. If evaporation rates are so great that the pellet becomes dry before E_3 and E_4 stages of embryonation are reached, the majority of the eggs die from desiccation. If, on the other hand, embryonation is rapid enough for the E_4 stage to be reached before complete drying occurs, the eggs can remain viable for a long period in a resistant dormant state. In studies, carried out under controlled conditions, on the evaporation of water from pellets, it was shown that virtually all the evaporable water was lost: within 24 hr. at 21·6 °C. and 3 days at 7·2° C. at 70 % R.H. and within 3 days at 21·6° C. and 9 days at 7·2° C. at 90 % R.H. These figures indicate how rapid loss of water can be even under comparatively mild drying conditions. Experiments are in progress to determine the water limits which control inhibition and hatching, and their effect on survival of populations.

Unhatched viable eggs have been kept at E_4 stage, in dried pellets at room temperature, for periods of up to 6 weeks. When moistened, such eggs hatch within a few minutes.

Hatching

Hatching occurs when the temperature is high enough (about 9° C.) to permit larval activity, and when eggs are in contact with water. Larvae generally emerge through a hole that has been produced by their sharp-pointed tail, and not by prodding through with their heads as described by Veglia (1915). The head prodding movement appears to be successful only if the aperture previously made by the tail is entered. The majority of larvae emerge tail-end first.

Larval development and migration

The results of observations on the rate of development of *H. contortus* from egg to the third larval stage are also summarized in Table 1. The data are again recorded as minimum and median times. The maximum time for development of any given population occurs several weeks after the median time.

It was found that larval production from a batch of pellets proceeds discontinuously for some considerable time, apparently depending on changing conditions in an ageing culture medium. Studies on the rate of larval production and migration from pellets were carried out on both individual and groups of pellets. Pellets were kept in corked tubes (3 in. × 3 in.) and transferred to fresh tubes either daily, or on every third day according to the temperature at which they were stored. The tubes in which the pellets had been kept were washed out and the rinse-water examined for larvae that had migrated from the pellet to the sides of the tube. Examples of the results obtained are summarized in Table 4 and Fig. 2. The results, at best, give only a broad indication of the rate of discontinuous development from material maintained at constant temperatures. Nevertheless, they would appear to be of sufficient value to indicate that the phenomenon is a significant one. Its extent and importance, however, needs to be determined, especially under natural climatic conditions.

Table 4. *Number of* Haemonchus contortus *larvae recovered at intervals; indicating rate of migration at two different temperatures* (11 *and* 14·4° C.) *from two cultures of eleven pellets each, raised at the same temperature* (14·4° C.)

Temp. (° C.)	Larval stages	Days 11	12	13	14	15	17	18	19	21	24	26	28
14·4	L_1	30	80	50	50	10	—	—	—	—	—	—	—
	L_2	60	470	90	210	40	—	20	20	—	330	—	—
	L_3	300	50	30	380	40	90	260	40	630	760	20	30
11	L_1	170	10	10	40	20	—	—	—	—	—	—	—
	L_2	640	90	280	180	10	—	60	20	20	—	90	—
	L_3	10	—	50	200	10	—	600	30	30	110	130	100

Temp. (° C.)	Larval stages	Days 31	33	35	38	40	42	45	47	49	52	54
14·4	L_1	—	—	—	—	—	—	—	—	—	—	—
	L_2	—	—	20	20	10	—	110	110	10	20	—
	L_3	10	130	10	30	20	20	100	30	10	—	20
11	L_1	—	—	—	—	10	—	—	—	—	—	—
	L_2	—	20	—	50	30	20	10	10	—	30	20
	L_3	30	30	—	130	190	180	70	10	—	30	—

Eggs and larvae of other species

Preliminary observations on *Ostertagia* spp., *Trichostrongylus* spp. and *Oesophagostomum venulosum* indicate that these worms have a substantially slower rate of embryonation than *H. contortus*, particularly at temperatures below 21·6° C. Moreover, they possess a greater degree of resistance to cold and desiccation and require almost twice as long as *H. contortus* for larval development at 14·4°, 11° and 7·2° C.

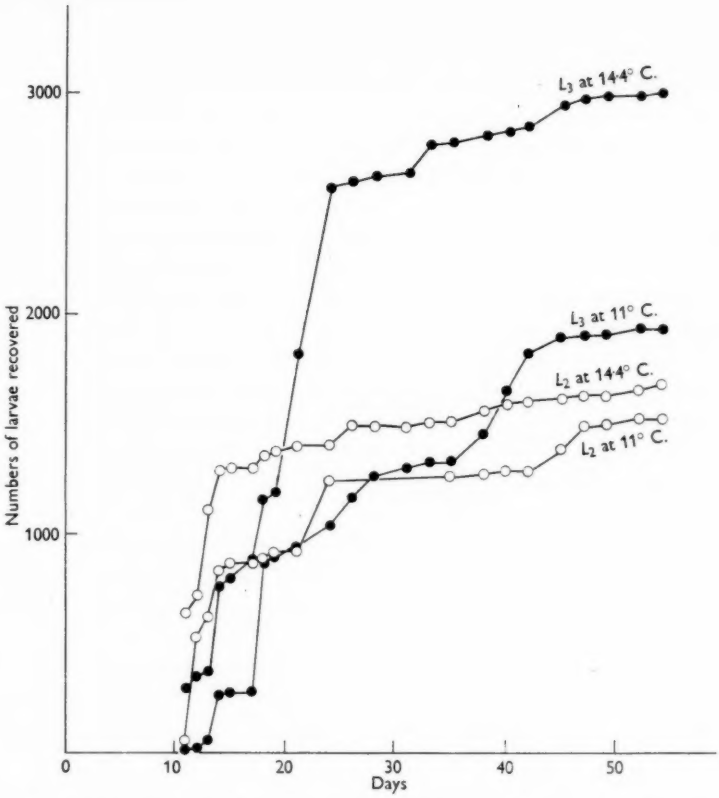

Fig. 2. Cumulative ogive indicating rate of migration of *Haemonchus contortus* larvae from faeces. Data from Table 4.

DISCUSSION

Monnig (1930) in South Africa found that *Trichostrongylus* spp. eggs containing complete embryos have a resistance much greater than that of infective sheathed larvae. He suggested that sheep could infect a pasture in the cold dry winter if the eggs could just reach the complete embryo stage, which may easily happen.

Crofton (1948*a*, *b*) demonstrated that eggs of the rabbit parasite *Trichostrongylus retortaeformis*, can overwinter in Britain. Eggs in faecal cultures which were placed on outdoor grass plots in November, December and January remained unhatched and viable at temperatures between 1·7° and 10° C. until April.

Gordon (1948) states that in *H. contortus* 'the embryonated egg and the infective larva are the more resistant stages'.

Our laboratory experiments show that *H. contortus* eggs resemble those of *Trichostrongylus* spp. in their ability to sustain an inhibition of development under adverse conditions, and to survive for long periods in the prehatch stage, and thus

support the views of Ransom (1906), Clunies Ross & Gordon (1936), and Gordon (1948).

The failure of Shorb (1943, 1944) and Dinaburg (1944) to obtain development or survival of *H. contortus* eggs at temperatures below 13° C. is difficult to understand since Ransom (1906) obtained results comparable with our own. It might be postulated that since Ransom's day the American strain of *H. contortus* has become physiologically different. It seems more likely, however, that many of the differences in results between Ransom and the present work on the one hand, and Shorb and Dinaburg on the other, are due to differences in technique. Ransom, like ourselves, cultured whole pellets. Shorb and Dinaburg ground and 'pulverized' pellets before culturing, and this could have altered conditions sufficiently to invalidate comparisons.

Our observations on factors affecting larval development agree with those made by Ransom in 1907. Larval development is strongly influenced by temperature, though moisture and aeration conditions may produce profound effects on the rate of growth. At a constant temperature of 21·7° C. and with adequate moisture and aeration, freshly passed *H. contortus* eggs require about 5 days before the first of them reach the third larval stage. It must be emphasized that the times quoted are minimal, and refer only to a very small proportion of any given population. Some larval development can proceed at a temperature as low as 7·2° C., but between this and 11° C. the bulk of a population is inhibited, the proportion of developing individuals increasing with increasing temperature.

To complete the picture of developmental relations, work remains to be done on the effect of temperature fluctuations. Nevertheless, it is of interest to relate the present results to the conditions which obtain in nature, where temperatures are considerably lower than the levels at which helminthologists are accustomed to rear their material. During a 5-year period (1943–7) of continuous temperature records taken under heather on a Scottish hill farm (600 ft. O.D.), the 10-day mean exceeded 15° C. on only one occasion (Campbell, 1948). The mean temperature over the 5-year period for the months June, July and August was 12·5° C. Air-temperature records from other parts of the United Kingdom are shown in Table 5.

Although the warm period of summer is the one when egg and larval development is most rapid, it is not to be expected that hot weather will inevitably result in more rapid turnover of populations. We have shown that an increase in temperature has a twofold effect: the first is direct acceleration of developmental rates, and the second is an indirect inhibition through desiccation. The net result clearly depends upon the time at which the drying process overtakes the development process.

The changes in the physical conditions of pellets take place more rapidly in the superficial than in the deeper parts. This has a differential effect on the developmental rates of contained nematode eggs according to their situation. These disparate conditions lead to an extension of the time limits of appearance of third-stage larvae. Even under relatively constant laboratory conditions this phenomenon is well marked, and larvae continue to appear over a considerable period of time. In the variable conditions which prevail out of doors it is to be expected

Table 5. *Examples of air temperature records* (° C.) *in different parts of the United Kingdom. Five-year means*

		May		June		July		August		September	
	Period	Mean	Mean max.	Mean	Mean max.	Mean	Mean max.	Mean	Mean max.	Mean	Mean max.
Scotland											
Craibstone (Aberdeenshire)	1943–7	8·9	12·7	11·7	15·6	13·9	17·8	13·4	17·5	11·9	15·3
	1951–5	8·6	12·2	11·2	14·8	13·7	17·9	13·3	16·9	11·3	14·8
Boghall (Midlothian)	1943–7	9·6	13·7	12·2	16·2	14·2	18·2	13·9	17·9	11·8	15·2
	1951–5	9·4	13·5	12·0	16·0	14·2	18·2	13·8	17·3	11·5	14·44
Peebles (Peeblesshire)	1943–7	9·2	13·7	12·0	16·4	14·2	18·5*	13·9	18·8*	11·3	15·7
	1951–5	9·4	14·2	11·9	16·6	14·0	18·9*	13·8	18·2	10·8	14·9
Peebles (under heather)	1943–7	9·0	12·1	12·1	14·4	13·3	16·0	13·1	15·6	9·6	12·4
England											
York (Yorkshire)	1943–7	12·0	16·9	14·4	18·9*	16·8	21·3*	16·4	21·1†	13·8	17·9
	1951–5	11·7	16·4	14·0	18·5*	16·3	20·9*	16·1	20·5*	13·6	17·7
Cambridge (Cambridgeshire)	1943–7	12·5	18·2	14·4	20·0*	17·2	22·2†	17·1	22·5†	14·3	19·0†
	1951–5	11·8	17·1	14·4	19·3*	16·9	21·9†	16·7	21·6†	13·8	18·5*
Wisley (Surrey)	1943–7	12·7	18·4*	15·0	20·0*	17·3	22·3†	17·2	22·5†	14·4	18·9*
	1951–5	12·2	17·0	14·8	19·7*	16·7	21·8†	16·7	21·1†	13·6	18·2
N. Ireland											
Hillsborough (County Down)	1951–5	10·1	13·8	12·1	15·6	13·9	17·8	14·3	18·0	11·7	15·0

* Above 'Dinaburg Line' of Kates (1950).
† Above embryonic developmental threshold of Dinaburg (1944).

that the spread of larval emergence is even more pronounced and more markedly discontinuous.

It is impotrant to note that sheep fed on grass regularly pass their faeces in the form of large aggregates of pellets. It is to be expected that the interior of such dung masses will have a greater inertia to climatic changes and, in particular, will dry up more slowly than discrete pellets under comparable conditions. This would result in an even further emphasis of the phenomenon of spread of larval emergence.

The factors affecting the migration of third-stage larvae have been investigated by several workers including Rogers (1940), Crofton (1948) and Rees (1950). In the present series of experiments both first- and second-stage larvae were observed to migrate from the pellets. The numbers of migrating first-stage larvae were very small, and are probably explicable on the basis of random movement. On the other hand, second-stage larvae migrated in substantial numbers, in some cases even exceeding the numbers of those which remained to develop to third stage (Fig. 2). This would seem to represent a real taxis which clearly requires further investigation.

The ability of infective larvae to survive under various climatic conditions has been studied by a number of workers in various countries with conflicting conclusions (see Kates, 1950). Most of the sampling techniques employed are designed to detect only third-stage larvae, and little or no account has been taken of the presence of unhatched eggs or of immature larvae. For example, Taylor (1957) reported the results of an experiment designed to compare the rate of survival of *H. contortus* in short and long grass. Each of eight plots, 9 in. square, was contaminated with 100 g. of sheep faeces in pellet form. Four of the plots had been cut short with scissors and the other four allowed to remain long. A ninth sample of 100 g. of the same faeces yielded 420,000 third-stage larvae out of an approximate 1,215,000 eggs in laboratory culture.*

Taylor writes: 'After allowing an interval of *three weeks*† for larval development successive plots were cut at weekly intervals; the larvae were then recovered by washing the grass in water and were enumerated under the microscope. The results are shown in the following table.' (Table 6.)

From these figures Taylor concluded: (1) that short grass is less favourable than long grass for survival of third-stage larvae, and (2) that about 600 eggs‡ are required to produce one infective larva (i.e. a mortality of 99·08 %). These conclusions are based on the assumption that non-recovery of individuals as third-stage larvae implies their death. This assumption is untenable in relation to these figures since the number of larvae recovered increases in each successive sampling week (i.e. there is here a paradox of mortalities decreasing as populations age). The experiments were carried out in October and November when field temperatures are comparatively low (they are unlikely to have exceeded a mean of 12° C.).

* It is of interest to note that the recovery rate here is approximately 30 % which might be expected for cultures reared at between 22° and 37° C.

† Our italics.

‡ This figure has been reached by taking the arithmetic mean of the 8 samples quoted in the table, i.e. 1869 larvae from 1,215,000 eggs, but since the samples are not comparable the procedure is invalid.

Under these conditions the 3-week interval allowed for larval development would be quite insufficient for more than the small minority of rapidly developing individuals. The bulk of the population would probably require a much longer time to complete development, and this view is amply supported by the trend of the figures for larvae recovered from long grass. Larval recoveries from short grass, except for the latest occasion, show the same trend, but they are consistently smaller. An increased mortality rate might be argued here but is unconvincing. There is no reason to suppose a more rapid development on these than on the long-grass plots, and a higher mortality in eggs at this time of year, which was stated to be 'very wet', seems unlikely. This result is difficult to understand unless some form of inhibition be postulated.

Table 6. (From Taylor, 1957.) *The numbers of third-stage larvae recovered from short grass and from long grass,* 4, 5, 6 *and* 7 *weeks after the deposition on to each plot of* 100 *g. of sheep's faeces containing* 1,215,000 *eggs of* Haemonchus contortus *per g.* [sic]

Date at which grass was cut (1954)	Number of third-stage larvae recovered		
	Short grass	Long grass	Proportionate number
18 October	19	12	$\frac{1}{2}$
25 October	316	1976	$\times 6$
1 November	465	3300	$\times 8$
8 November	19	8487	$\times 400$

Our findings on the relationship between temperature and developmental rates differ from those of Veglia in South Africa and Shorb and Dinaburg in North America. This is most pronounced when developmental threshold levels and developmental rates in the lower levels of the biokinetic range are considered. It is possible that our strain of *H. contortus* is physiologically different from the North American and South African strains, but confirmation of this point would require a proper comparative study. At least, it must be concluded that the application of results derived from North American or South African experience must be applied to British conditions with caution. Even the most cursory reference to the temperature conditions which prevail in these islands (Table 5) must compel the realization of the complete inapplicability of the 'Dinaburg line' in the interpretation of worm outbreaks here. Equally obvious is the fallacy of the assumption that the 3- to 6-day cycle, which is frequently quoted (Spedding, 1954*a*; Lapage, 1956), could suffice for completion of development from egg to infective larva. In British field conditions the evolution of infective larvae of *H. contortus* must require upwards of 2 weeks in summer, and considerably more at other times of the year. From preliminary studies on other strongyloid species, we have evidence that their cycle of development is even more prolonged, and that their ability to endure adverse conditions is even better developed.

For these reasons, we find it impossible to support the recommendation (Spedding, 1954*a*, *b*) that a laborious strip grazing system, involving movement of

animals every second day, is necessary to prevent re-infestation with parasitic worms. The advice (National Agricultural Advisory Service, and Thomson & Corner, 1957) that paddocks be grazed for 5 days or less and the animals returned after 25–30 days would appear equally to be founded upon an unsound rationale. Indeed, the proposition that animals be returned to re-graze after 4 weeks or so may be a dangerous one since their return, during the summer months, would coincide with the peak of larval production. We believe the pattern of helminth population dynamics is of such complexity that a detailed definition of optimal times for grazing and re-grazing is premature. For example, during summer, initial grazing periods could safely range from 2 to 6 weeks depending upon the temperature record over the period. Unfortunately the requisite rest period to guarantee non-infestation thereafter would require to be considerably longer than is at present generally recognized, and most likely would have to extend into the following season. In relation to the problem of the proper use of grass this proposition becomes unrealistic.

SUMMARY

Laboratory studies on the embryonic and larval development of *Haemonchus contortus* under constant temperatures of 0·0°, 7·2,° 11°, 14·4°, 21·7° and 37° C., and varying moisture conditions were carried out. Reports of preliminary studies on *Ostertagia* spp., *Trichostrongylus* spp. and *Oesophagostomum venulosum* are included.

Observations showed that eggs in the same culture may behave in several ways: (1) eggs may develop at different rates; (2) eggs may exhibit intermittent stages of active and inhibited development; and (3) eggs may fail to develop and die at any stage from unknown causes.

At a constant temperature of 21·7° C. and with adequate moisture and aeration, freshly passed *H. contortus* eggs require 5 days before the first of them reach the third larval stage. At 14·4° C. it takes 9 days, whilst at 11° C. at least 15 days must pass before third-stage larvae first appear. Some larval development can proceed at a temperature as low as 7·2° C.

Mortality and inhibition vary with temperature. The proportion of eggs which survives to the prehatch stage varies between 10 and 90% with a maximum survival at 11° and 14·4° C. Embryonated eggs are found to be very much more resistant to adverse conditions than unembryonated eggs. Embryonated eggs survive at 7·2° C. for up to 4 months.

Excess water was found to inhibit development, probably owing to its interference with aeration. The period of survival of eggs in saturated faecal cultures maintained at different temperatures was determined.

Rapid desiccation of faecal pellets resulted in the destruction of the contained nematode eggs unless the eggs were in an advanced stage of embryonation. Eggs in the prehatch stage were found to be extremely resistant and were kept in a dry condition for up to 6 weeks in an unhatched though viable state.

Owing to the disparate conditions in faecal cultures which result in varying

degrees of inhibition, it was found that there is a discontinuous development of first-, second- and third-stage larvae over a considerable period of time.

The relation of these laboratory observations to the conditions occurring in the field are discussed, and it is concluded that *H. contortus* requires about 2 weeks in the summer for development from egg to third-stage larva and considerably longer periods are needed at other times of the year.

From preliminary studies on other strongyloid species, the evidence indicates that their cycle of development is even more prolonged and that their ability to endure adverse conditions is better developed.

We wish to record our indebtedness and thanks to Miss Sandra Dunbar, M.I.S.T., for technical assistance. The meteorological data were obtained through the courtesy of the Agricultural Research Institute of Northern Ireland and the Meteorological Office, Edinburgh.

REFERENCES

Campbell, J. A. (1948). The life history and development of the sheep tick *Ixodes ricinus* L. in Scotland under natural and controlled conditions. Ph.D. thesis, University of Edinburgh.

Crofton, H. D. (1948*a*). The ecology of immature phases of Trichostrongyle nematodes. I. The vertical distribution of infective larvae of *Trichostrongylus retortaeformis* in relation to their habitat. *Parasitology*, **39**, 17–25.

Crofton, H. D. (1948*b*). The ecology of immature phases of Trichostrongyle nematodes. II. The effect of climatic factors on the availability of the infective larvae of *Trichostrongylus retortaeformis* to the host. *Parasitology*, **39**, 26–38.

Cunliffe, G. & Crofton, H. D. (1953). Egg sizes and differential egg counts in relation to sheep nematodes. *Parasitology*, **43**, 275–86.

Dinaburg, A. G. (1944). Development and survival under outdoor conditions of eggs and larvae of the common ruminant stomach worm, *Haemonchus contortus*. *J. Agric. Res.* **69**, 421–33.

Gordon, H. McL. (1948). The epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep. *Aust. Vet. J.* **24**, 17–45.

Kates, K. C. (1950). Survival on pasture of free-living stages of some common gastrointestinal nematodes of sheep. *Proc. Helm. Soc. Wash.* **17**, 39–58.

Lapage, G. (1956). *Veterinary Parasitology*. Edinburgh: Oliver & Boyd.

Monnig, H. O. (1930). Studies on the bionomics of the free-living stages of *Trichostrongylus* spp. and other parasitic nematodes. *Rep. Vet. Res. S. Afr.* **16**, 175–98.

Ransom, B. H. (1906). The life history of the twisted wireworm (*Haemonchus contortus*) of sheep and other ruminants. *Circ. U.S. Bur. Anim. Ind.* **93**, 7 pp.

Ransom, B. H. (1907). Stomach worms (*Haemonchus contortus*) in sheep. *Circ. U.S. Bur. Anim. Ind.* **102**, 7 pp.

Rees, G. (1950). Observations on the vertical migrations of the third-stage larva of *Haemonchus contortus* (Rud.) on experimental plots of *Lolium perenne* S 24, in relation to meteorological and micrometeorological factors. *Parasitology*, **40**, 127–43.

Rogers, W. P. (1940). The effect of environmental conditions on the accessibility of third-stage trichostrongyle larvae to grazing animals. *Parasitology*, **32**, 208–25.

Ross, I. Clunies & Gordon, H. McL. (1936). *The Internal Parasites and Parasitic Diseases of Sheep*. Sydney: Angus & Robertson.

Shorb, D. A. (1943). Survival on grass plots of eggs and pre-infective larvae of the common sheep stomach worm *Haemonchus contortus*. *J. Parasit.* **29**, 284–9.

Shorb, D. A. (1944). Factors influencing embryonation and survival of eggs of the stomach worm *Haemonchus contortus*. *J. Agric. Res.* **69**, 279–87.

Spedding, C. R. W. (1954*a*). Production of worm-free lambs at pasture. *Nature, Lond.*, **174**, 611.

SPEDDING, C. R. W. (1954*b*). Pasture management to control worms in sheep. *Agriculture, Lond.*, **61**, 51–4.

TAYLOR, E. L. (1957). An account of the gain and loss of the infective larvae of parasitic nematodes in pastures. *Vet. Rec.* **69**, 557–63.

THOMSON, D. & CORNER, H. H. (1957). Rotational grazing of ewes and lambs in Roxburghshire. *Scot. Agric.*, pp. 205–7.

VEGLIA, F. (1915). The anatomy and life history of the *Haemonchus contortus* (Rud.). *Rep. Vet. Res. S. Afr.* **3**, **4**, 349–500.

WHITLOCK, H. V. (1957). Personal communication, McMaster Laboratory C.S.I.R.O., Parramatta Road, Glebe, N.S.W.

(*MS. received for publication* 22. XI. 1957.—Ed.)

THE ANATOMY OF *KÖLLIKERIA FILICOLLIS* (RUDOLPHI, 1819), COBBOLD, 1860 (TREMATODA: DIGENEA) SHOWING THAT THE SEXES ARE NOT ENTIRELY SEPARATE AS HITHERTO BELIEVED

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(With 17 Figures in the Text)

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I. INTRODUCTION

There are over sixty species in the trematode family Didymozoidae Poche, 1907, one of which, namely *Köllikeria filicollis* (Rudolphi, 1819), Cobbold, 1860, is described in detail below. The following quotations from Stunkard (1946) and Dawes (1946) give an indication of the state of our knowledge of the family. Stunkard stated that it 'contains aberrant forms, in part cyst-dwelling and in part dioecious, which parasitize marine fishes and whose systematic position remains problematical. Some are monostomes, others distomes, and although the family was monographed by Dollfus (1926) and Ishii (1935), neither author attempted to relate them to other Digenea. Indeed, Ishii's account of the life history, brief and probably not correct, predicted direct, i.e. monogenetic development.' Dawes was of the opinion that 'early studies were incomplete and our knowledge is still limited, our ignorance profound, on the structure of forms well known by name'. As *K. filicollis* is a little-known species a detailed description of its anatomy may contribute towards clearing some of the confusion that exists regarding the classification of the family Didymozoidae. The study has revealed that the sexes of *K. filicollis* are not entirely separate as hitherto believed. Nevertheless, what

have been regarded previously as distinct males and females will, for convenience, be referred to as 'males' and 'females' in the description below.

The material for the investigation was obtained on one of ten specimens of *Brama raii* (Bloch) trawled from about 191 fathoms at latitude 59° 10′ N. and longitude 6° 40′ W. by the Fleetwood motor-trawler *Irvana*. Temporary mounts of specimens fixed in 5% formaldehyde were examined under a coverslip in water, lactophenol or a solution of glycerine in alcohol. For permanent whole mounts Ehrlich's haematoxylin was used as stain, while serial sections cut in three planes, transverse, horizontal and sagittal, were stained with Ehrlich's haematoxylin and eosin.

II. EXTERNAL FEATURES

K. filicollis is, like most species of the Didymozoidae, a cyst-dwelling form. Four elliptical orange-yellow cysts (Fig. 1) were found attached to the epithelium inside the operculum near the posterior gill of one *B. raii* (Bloch). Each cyst is about 9·10 mm. long, 6·50 mm. broad and 2·5 mm. deep, and contains from one to five 'males' (Fig. 5) and one 'female' (Fig. 2), a total of thirteen 'males' being found in the four cysts. The 'male' worm is greyish white in colour, filiform (Fig. 5), and is 13·50–17·00 mm. long and 0·41–0·78 mm. in maximum breadth midway between the oral and ventral suckers. The oral sucker is rounded, 0·15–0·47 mm. broad, subterminal and on the ventral surface. The ventral sucker is 0·41–0·78 mm. behind the oral sucker and is 0·28–0·31 mm. broad. The body of the 'female' worm (Fig. 2) is divided into two regions, the fore- and the hind-body. The fore-body is almost identical with the anterior region of the 'male' and is 5·5–7·2 mm. long and 0·74–1·20 mm. broad midway between the oral and ventral suckers. The oral sucker is 0·44–0·50 mm. broad, while the ventral sucker is 0·29–0·30 mm. The hind-body of the female is swollen and curved upon itself to become roughly kidney shaped. On its ventral side and extending along the median line there is a shallow groove (Figs. 11, 13). At the posterior end of this groove (Fig. 2) and on either side there is a conical projection which is 0·17 mm long and 0·25 mm. broad at the base. It has been suggested by previous workers that the groove and the two projections on the ventral surface of the 'females' of members of the genus *Köllikeria* may serve the function of holding the male worm in position during copulation. The writer is unable to confirm this as no 'males' were found in the groove.

III. INTEGUMENT, MUSCULATURE AND THE CYST

The writer is not aware of an account of the body wall and the cyst in members of the family Didymozoidae although, as has already been stated, most of the sixty known species are cyst dwellers. In the case of *K. filicollis* an examination of the cyst has shown that it is mainly a reaction product of the host's tissue against secretions from some of the subcuticular gland cells of the 'female' worm. The integument, musculature and the structure of the cyst will be described below and this will be immediately followed by a general discussion on the body wall in some other digenetic trematodes. The cuticle (Fig. 14), which rests on a basement

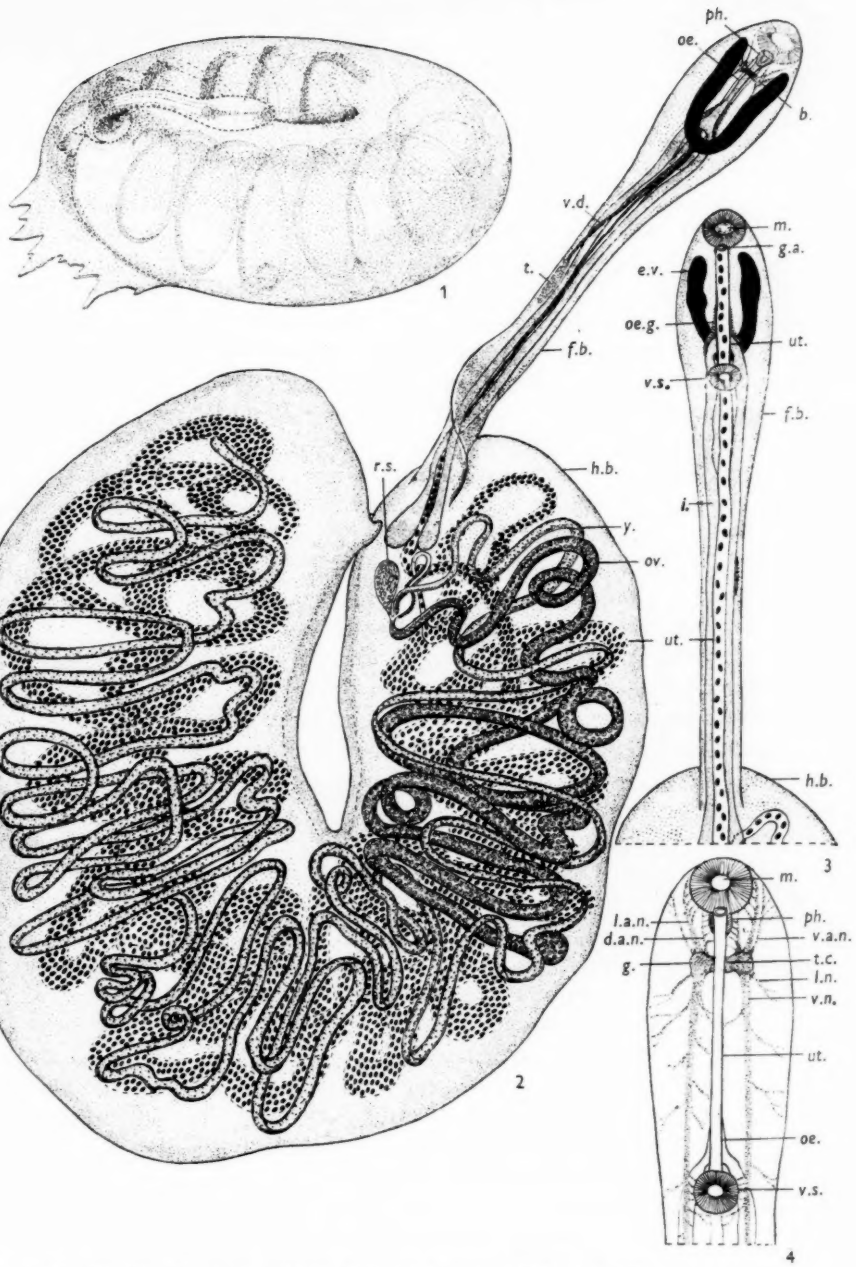


Fig. 1. *K. filicollis*, showing position of 'female' worm within the cyst.
Fig. 2. 'Female' showing hind-body in dorso-lateral view and the fore-body in dorsal view.
Fig. 3. 'Female' fore-body and part of hind-body in ventral view.
Fig. 4. 'Female' in ventral view showing nervous system.

membrane, varies in depth from 0·003 mm. in the fore-body of both 'males' and 'females' to only 0·0015 mm. in the hind-body of the 'female.' The cuticle is followed by the basement membrane and the musculature of the body wall. This latter consists of a single layer of delicate circular muscle fibres, each fibre being 0·0015 mm. in diameter. The longitudinal muscle fibres are even more delicate, many fine strands occupying a depth of only 0·0015 mm. Dorso-ventral muscles occur very spasmodically throughout the length of the body and can only be seen clearly in the regions of the oral and ventral suckers. Subcuticular cells which are of two kinds are well developed in both 'males' and 'females', and in particular in the dorsal wall of the hind-body of the 'female'. Immediately beneath the musculature (Fig. 14) there is a single layer of regularly spaced subcuticular gland cells, each of which possesses very granular cytoplasm and a relatively small nucleus 0·003 mm. in diameter. This layer of cells is followed by another layer of irregularly arranged cells of a different kind whose nuclei are relatively much larger, being 0·006 mm. in diameter. The parenchymatous cells that are present throughout the body of the parasite can be distinguished from the two kinds of subcuticular cells by their very granular nuclei and less granular cytoplasm.

The single layer of subcuticular cells immediately beneath the musculature appears to be concerned with cyst formation (Fig. 14). These cells exude their granular contents through minute pores in the cuticle, the secretion forming a layer 0·003 mm. deep surrounding the parasite. The secretion which is the innermost layer of the cyst is in very close association with the host's epithelial tissue, which appears to have degenerated to form the outer layer of the cyst. This degeneration of the host's epithelial tissue is probably the result of its reaction against the glandular secretion of the parasite. Groups of from two to fifty flattened, oval, nucleated cells can be seen at regular intervals to be closely associated with the inner layer of the cyst. Whether these groups of oval cells are degenerate macrophages, lymphocytes or host epithelial cells is impossible to ascertain. The oval cells are embedded in fibres of loose vacuolated connective tissue in which are scattered small nuclei and large darkly stained rounded bodies, possibly melanophores. The irregularly arranged layer of subcuticular cells with the relatively large nuclei may be myoblasts, as there is slight evidence that the longitudinal and circular muscles of the body wall are products of these cells.

Well-developed subcuticular cells similar to those described above are present in many digenetic trematodes and have been investigated by several workers. Odlaug (1948) gave a detailed account of previous work on the subject when discussing the finer structure of the body wall and the parenchyma of three species of digenetic trematodes, one of which, namely *Loxogenes arcanum*, is a cyst dweller. Odlaug described four types of subcuticular cells in the three species and stated that only *L. arcanum* which occurs encysted in the duodenum and pyloric end of the stomach of the frog possesses a 'true layer of subcuticular cells', but he did not, however, attribute any particular function to those cells. He stated that there is no correlation between the structure of the cuticle and the environment of the three parasites he studied. Pratt (1909) was of the same opinion, and stated that the structure of the cuticle is not a significant factor in the resistance

of the parasite to its environment, but that chemical secretions of the subcuticular cells are more important. The present investigation into the structure of the body wall and cyst of *K. filicollis* supports the view of Pratt (1909) and Odlaug (1948) that the cuticle in some digenetic trematodes cannot be correlated with its habitat. *K. filicollis* appears to derive its protection by the formation of a cyst which is the product of the reaction of the host's tissue to a secretion from subcuticular gland cells, this secretion forming the innermost layer of the cyst. This is interesting when compared with the work of Hunter & Hamilton (1941), who described cysts in their studies on host reactions against larval parasites of the families Strigeidae, Clinostomatidae and Cryptocotylidae. They stated that the host tissues reacted by accumulating cells, apparently macrophages and lymphocytes, on the outside of an inner hyaline cyst. This kind of reaction, therefore, appears to be identical with that of *B. raii* against *K. filicollis*. Hunter & Hamilton, however, stated that 'this inner hyaline cyst is believed by parasitologists to be of parasitic origin and there is no evidence to the contrary'. In *K. filicollis* the inner layer of the cyst is definitely of parasitic origin and, as has already been stated, it is derived from subcuticular gland cells which discharge their products through minute pores in the cuticle. Many stages in the discharge of the contents of the subcuticular gland cells were seen by the writer (Fig. 14). Some of the cells were full of granular material, others showed their contents in the process of being discharged, while some were empty. In some cases the nucleus of the cell was lodged in the cuticular pore, appearing at first sight to be embedded in the cuticle. This is interesting, as according to Dawes (1946) Monticelli thought that the cuticle of digenetic trematodes was a metamorphosed epithelium which had suffered chemical alteration of its protoplasm and the loss of its nuclei, this being supported by the fact that in some larval and adult Digenea the cuticle contains nuclei, or bodies which resemble nuclei. It may be possible that Monticelli was mistaken in his views and that the nuclei seen in the cuticle are, as in *K. filicollis*, products of the subcuticular cells that have become lodged in minute cuticular pores.

The deeper situated subcuticular cells in *K. filicollis* may be associated with the formation of circular and longitudinal muscle fibres. Dawes (1946) stated that myoblasts sometimes occurred beneath the general musculature of digenetic trematodes. It is interesting to observe that in *K. filicollis* the musculature is well developed where the deeper seated subcuticular cells are not well developed and vice versa. For instance, in the hind-body of the female this layer of subcuticular cells is very well developed, but the musculature is hardly discernible. It may be that many cyst-dwelling parasites, whose movements are restricted, have prominent subcuticular cells, as the myoblasts are not drawn upon extensively to form the musculature.

IV. ALIMENTARY CANAL

The alimentary canal of the 'male' and 'female' worms will be described separately as there are slight differences.

In the 'male' the ventral mouth (Figs. 5, 10) opens through the centre of the oral sucker into the dorsally situated muscular pharynx which is 0·15–0·25 mm.

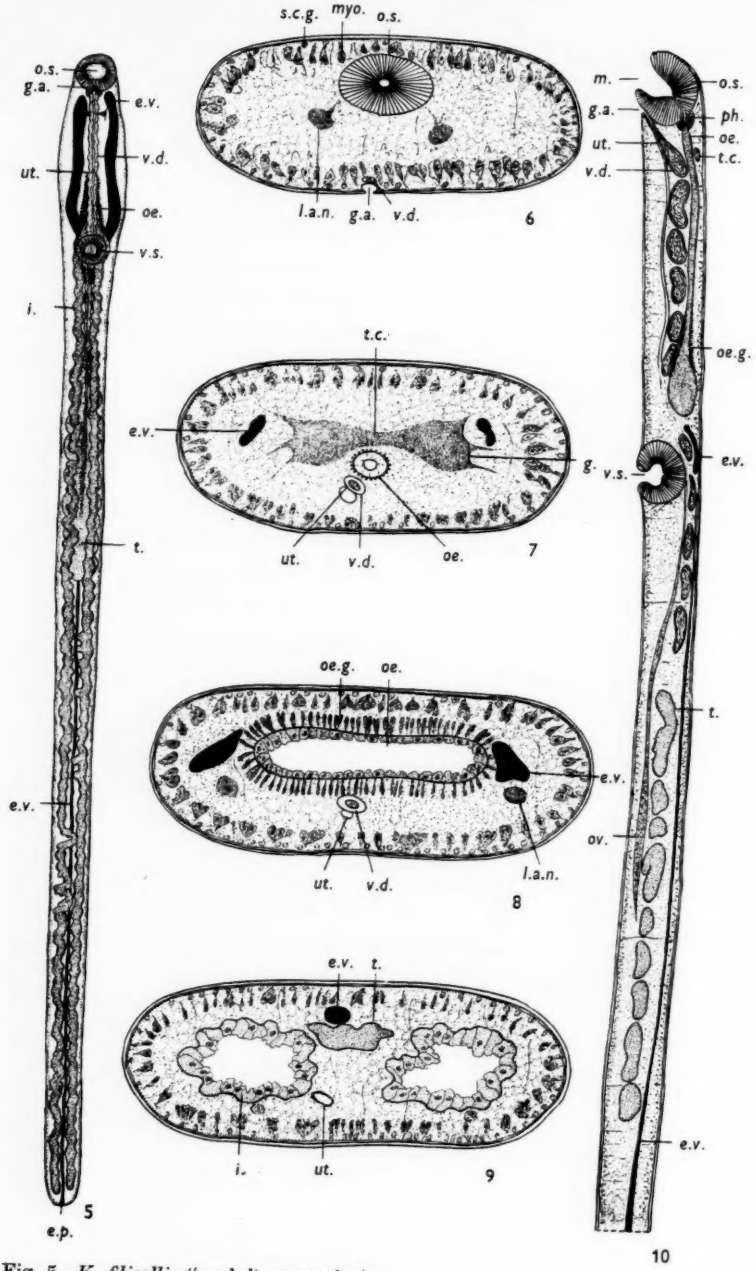


Fig. 5. *K. filicollis* ('male'), ventral view.
Fig. 6. Transverse section through genital atrium.
Fig. 7. Transverse section through brain.
Fig. 8. Transverse section through dilation of oesophagus and excretory vessels.
Fig. 9. Transverse section through testes.
Fig. 10. Median sagittal section through anterior region.

long and 0·08–0·10 mm. broad. The wall of the pharynx is 0·054 mm. thick and its lumen 0·016 mm. in diameter. Following the pharynx is the oesophagus which is 0·40–1·22 mm. in length and has a uniform diameter of 0·07 mm. for about the first two-thirds of its length, but is dilated for the last third (Fig. 8) to a diameter of 0·09–0·10 mm. The oesophagus then bifurcates slightly in front of the ventral sucker (Figs. 5, 10) to form the intestinal caeca which extend backwards to terminate within a short distance of the posterior end of the body. The intestinal caeca which have a uniform diameter of 0·17 mm. throughout their lengths are thrown into numerous undulations (Fig. 5). There are conspicuous circular and longitudinal muscles in the walls of the caeca surrounding their very well-developed epithelial lining (Fig. 9). Gland cells occur in the region of the intestinal bifurcation (Figs. 8, 10), extending for a short distance forwards around the oesophagus and backwards around the intestinal caeca. The contents of these cells are granular and identical granules were found in the lumen of the gut in these regions.

The alimentary canal of the 'female' (Figs. 2, 3) is very similar to that of the male, differing mainly in the relative length of the intestinal caeca, which extend only for a short distance into the hind-body. The caeca also differ from those of the 'male' in that they are fairly straight and their terminations are club-shaped (Fig. 2).

V. EXCRETORY SYSTEM

Very little is known about the excretory system in members of the family Didymozoidae. According to Yamaguti (1938), in some members of the genus *Didymocystis* Ariola, 1902, there is a median ventral excretory vessel bifurcating anteriorly in front of the bifurcation of the intestine into two short arms. In general terms the excretory system is the same in *K. filicollis* but there are a few differences. In the 'male' there is an excretory vessel in the mid-dorsal line and this opens to the exterior at the posterior extremity of the body (Fig. 5). The excretory vessel extends forwards to the region of the ventral sucker (Fig. 10) and bifurcates here on to lateral dilated vessels (Figs. 5–8) which extend forwards as far as the lateral margins of the oral sucker. The median dorsal excretory vessel is about 0·123 mm. in diameter throughout its length, having slightly muscular walls, its lumen containing finely granular material. The anterior lateral dilatations of this vessel are about 0·500 mm. in diameter, having thin walls containing small nuclei, and are without granular material in the lumen.

In the fore-body of the 'female' (Figs. 2, 3) the excretory system is like that of the 'male' being Y-shaped, with the stem of the Y extending as far as the hind-body; no trace of the excretory system could be found in the hind-body.

VI. NERVOUS SYSTEM

The previously undescribed nervous system of *K. filicollis* consists of a 'brain' (Figs. 2, 4 and 7) situated dorsally to the oesophagus giving off anterior and posterior nerves.

The 'brain', which is about 0·382 mm. behind the posterior border of the pharynx,

is composed of two ganglia joined by a transverse commissure. Each ganglion is about 0·15 mm. in diameter and gives off three nerves anteriorly, the dorsal, the lateral and the ventral anterior nerves (Fig. 4). The dorsal anterior nerves supply the pharynx, the lateral anterior nerves the oral sucker while the ventral anterior nerves innervate the body wall. From each cerebral ganglion there arises posteriorly a very short lateral nerve and a ventral nerve, which extend backwards as far as the posterior end of the body in the 'male' but could not be traced beyond the posterior end of the fore-body in the 'female'. The two short lateral nerves supply the body wall, but the two ventral nerves, which are much branched, supply the body wall, the ventral sucker, the intestinal caeca and the genitalia.

VII. GENITALIA

Hitherto it was thought that the sexes were entirely separate in all members of the genus *Köllikeria*, but this study has revealed rudiments of the male organs in the 'female' and female organs in the 'male'. The proximal ends of both male and female ducts open into a shallow genital atrium which is situated in the mid-ventral line immediately behind the oral sucker (Figs. 3, 5 and 10).

(*a*) *Male genitalia*

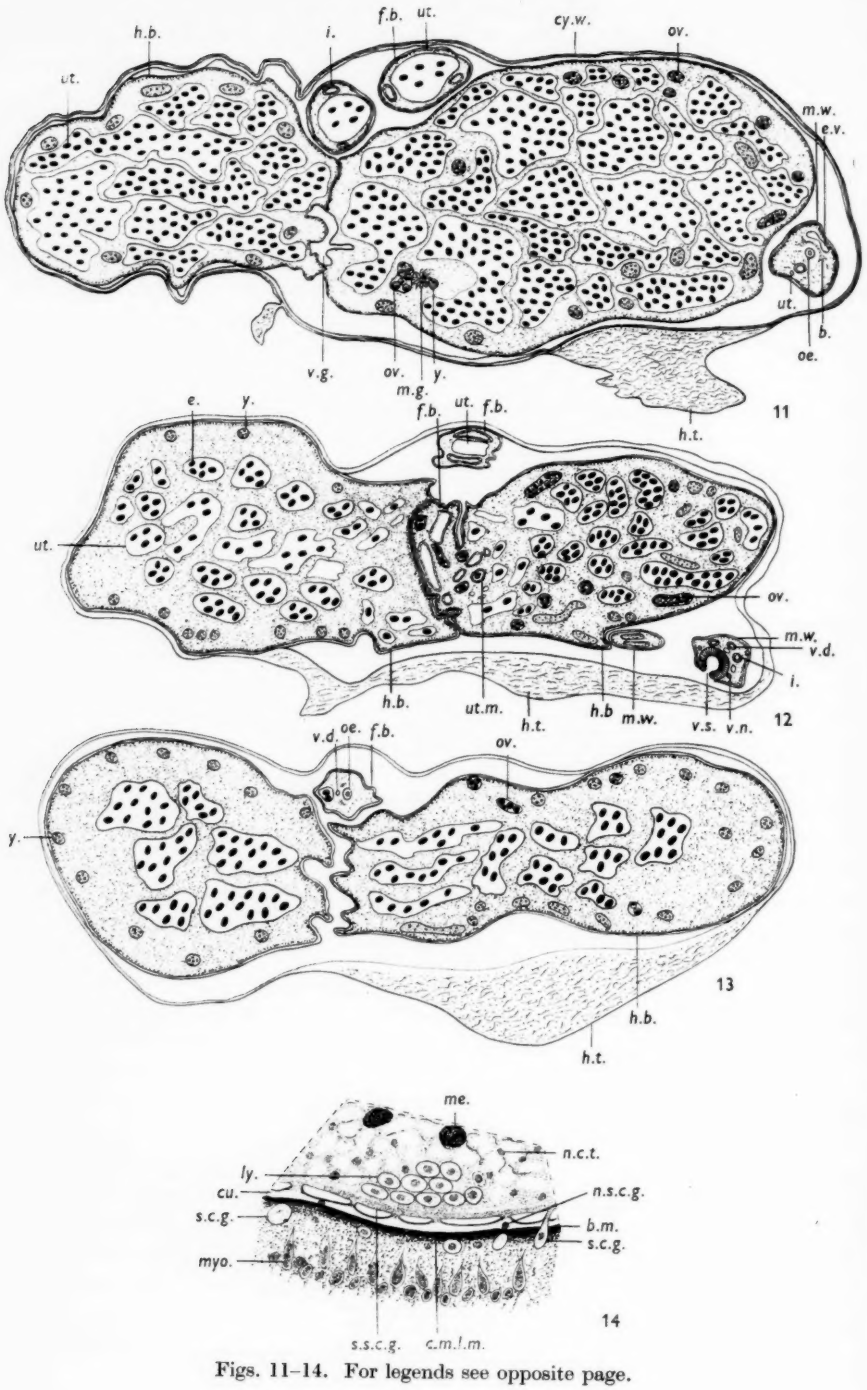
The male genitalia are fully developed only in the 'male' worms (Figs. 5, 9 and 10). The single, much convoluted testis which is 11–12 mm. in length is situated in the dorsal half of the body (Figs. 5, 9 and 10) its anterior border being a short distance behind the ventral sucker. The vas deferens (Figs. 5, 10) arises from the testis antero-dorsally, extending forwards in the dorsal half of the body as a thin tube in a slightly convoluted course. It has a somewhat muscular wall and is 0·015 mm. in diameter. In front of the ventral sucker the vas deferens becomes more ventral in position (Fig. 10), continuing forwards below the oesophagus to open into a very shallow genital atrium. Proximally the vas deferens may or may not be dilated to a diameter of 0·083 mm., being filled with spermatozoa and functioning presumably as the vesicula seminalis. There is no cirrus.

Rudiments of the testis and vas deferens occur in the fore-body of the 'female' (Fig. 2). The testis is in the form of undifferentiated tissue which contains nuclei and which is situated dorsally or laterally to the uterus half-way along the length of the fore-body. From its anterior region arises a rod of similar tissue which extends forwards below the uterus as far as the genital atrium.

(*b*) *Female genitalia*

The ovary, which is in the hind-body (Fig. 2) is elongated, slender and convoluted, having a diameter of about 0·050 mm. throughout its length. The distal end of the ovary is slightly swollen, being roughly club-shaped, and is situated dorso-laterally beneath the body wall at about a third of the length along the hind-body. From here it extends forwards, coiling below the body wall (Figs. 2 and 11–13) to end ventrally near the left side of the hind-body (Figs. 2, 11). The oviduct arises from the proximal end of the ovary, receiving first on its outer side (Figs. 2, 15) the duct

of the receptaculum seminis and close to it the yolk duct. The oviduct continues antero-dorsally, opening into the central chamber, which is 0·25 mm. long and 0·33 mm. broad. The central chamber is surrounded by Mehlis's gland cells, the basal portions of these cells containing nuclei and staining a light blue with Ehrlich's haematoxylin, while the distal portions of the cells are without nuclei and stained a light red with eosin. The central chamber is continuous with the thin-walled uterus which at first runs forward as far as the distal ends of the intestinal caeca, describes a loop postero-dorsally and extends as the much-coiled descending limb as far as the posterior end of the hind-body. From here it runs forward again as the ascending limb which is not so coiled and which near the anterior end of the hind-body becomes thick-walled and muscular (Figs. 12, 17). This muscular region leads into the thin-walled distal end of the uterus which enters the fore-body and takes a straight mid-ventral course to the genital atrium. The function of the muscular portion of the uterus may be to force eggs into that part of the uterus occupying the fore-body, the extreme length of the uterus and the enormous number of eggs produced perhaps necessitating a 'pumping mechanism' of this kind. The proximal end of the uterus near the central chamber is surrounded for some of its length (Fig. 15) by small glandular cells which appear to be different from those of Mehlis's gland cells. The receptaculum seminis (Figs. 2, 15) is a swollen pear-shaped sac measuring 0·29 mm. in length, 0·15 mm. in greatest breadth and is distended with yolk cells and spermatozoa. The yolk gland, which is elongated and coiled beneath the body wall, extends from its junction with the oviduct to the posterior end of the hind-body. Distally near the central chamber the yolk gland is slightly swollen to form the yolk reservoir which is 0·25 mm. long and 0·082 mm. broad. The eggs are exceedingly numerous and oval in shape measuring 0·021 × 0·016 mm. in the proximal region of the uterus and increasing in size to 0·025 × 0·17 mm. in its distal region. The thickness of the shell of those eggs in the distal end of the uterus is also greater than that of the eggs seen in the proximal region, the range being 0·0008–0·001 mm. There is an operculum at one pole of the egg (Fig. 16) and each egg contains a well-developed embryo by the time it reaches the distal end of the uterus. There is a crown of fine spines around a conical projection at the opercular end of the embryo, but these are too small for their exact shape and size to be determined. A similar crown of spines has been observed by Wagner (1858), Baylis (1938) and Nigrelli (1939) in members of the genera *Didymozoon* and *Didymocystis*. From serial sections the following observations have been made on the egg and the egg shell, and these are presumably various stages in their formation. In some sections ova and groups of yolk cells, each of these latter containing clusters of rounded granules, presumably of polyphenol, were seen in the central chamber. In others showing the proximal part of the uterus the yolk cells were either arranged in a single row, together with an ovum, or this latter was surrounded by a group of up to twenty-five yolk cells which were in turn surrounded by spermatozoa. Polyphenol granules also occurred outside the group of yolk cells at this stage, but whether this was due to mechanical injury is not known. Other stages either showed the ovum orientated at one end of many broken-down yolk cells and masses of unevenly distributed polyphenol granules or these latter were



Figs. 11–14. For legends see opposite page.

arranged in a layer of equal depth surrounding the broken-down yolk cells. A very thin egg shell was always present around the layer of polyphenol granules. The present observations agree in general with the view of Nigrelli (1939) on egg-shell formation in *Didymocystis coatesi*. They also agree with the findings of Stephenson (1947) and others, who have stated that the egg shell of trematodes, and in particular that of *Fasciola hepatica*, originates from granules present in the vitelline cells and not from a secretion of Mehlis's glands, this secretion being reinforced by the granules of the vitelline cells. On the other hand, it is not in agreement with the views of Yosufzai (1953) and others. According to Yosufzai (1953) the egg of *F. hepatica* is first formed when it passes out of the central chamber, and he stated that the hyaline secretion of Mehlis's glands which forms the outer shell of the egg of this species has a marked affinity for eosin. In *Köllikeria filicollis* the first indication of the egg shell being present is about 1·25 mm. from the central chamber, and although the present specimens were stained in haematoxylin and eosin the egg shell when it was first apparent had no affinity for eosin. It is possible, however, that the glandular cells surrounding the proximal part of the uterus near the central chamber may play a part in the formation of the egg shell, as it is in this region that most of the above-described stages were observed.

Rudiments of the female genitalia occur in the 'male' worms (Fig. 10). There is a well-defined mass of nucleated tissue ventral to the testis, and from it there extends forwards a rod of undifferentiated tissue containing nuclei or in some cases a duct, both of which take a course below the vas deferens to open into the genital atrium. According to Yamaguti (1934) rudiments of the male and female genitalia in the 'female' and 'male' worms respectively occur in *Wedlia orientalis*. The writer's description of these genital rudiments agrees closely with Yamaguti's observations. Yamaguti (1953) placed the species *Wedlia orientalis* in the genus *Köllikeria*.

VIII. THE SYSTEMATIC POSITION OF *KÖLLIKERIA FILICOLLIS*

Ishii (1935) divided the family Didymozoidae Poche, 1907, into many subfamilies, but his scheme of classification is not generally accepted because, as Baylis (1938) has pointed out, Ishii's subfamilies are based upon unsatisfactory characters. He distinguished some of his subfamilies on whether or not the sexes were entirely separate, for example he stated that they were separate in Köllikeriinae but not

Fig. 11. *K. filicollis*, transverse section through cyst, showing 'male' and 'female' worms in transverse section. The section through the hind-body of the 'female' shows the proximal end of the ovary and yolk gland. The transverse section of the 'male' shows the brain.

Fig. 12. Transverse section through the cyst, showing 'male' and 'female' in transverse section. This latter shows the fore-body of the 'female' emerging from the hind-body. The section of the 'male' is through the ventral sucker.

Fig. 13. Transverse section through cyst, showing 'female' in transverse section. The section, which is through the region of the distal end of the ovary, shows the ventral groove in the hind-body.

Fig. 14. Detailed drawing of the body wall and part of the cyst wall.

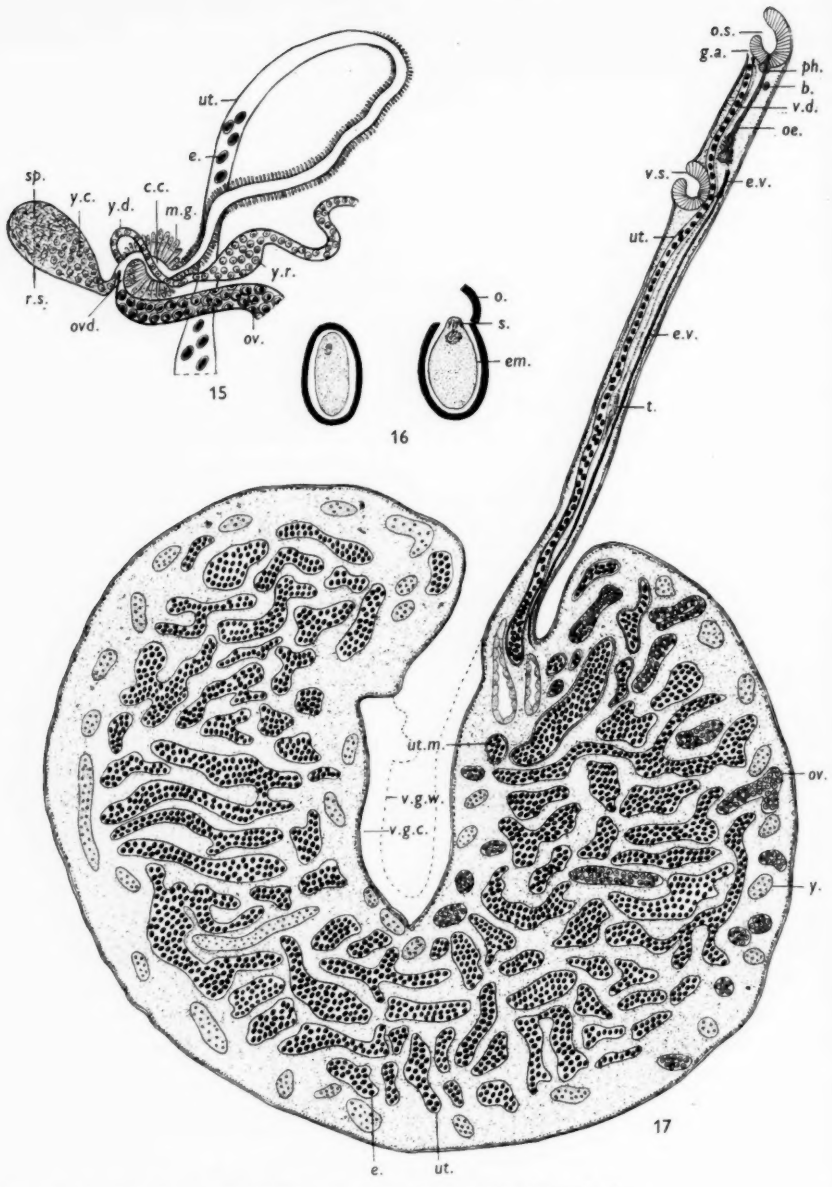


Fig. 15. *K. filicollis*. Reconstruction of female genitalia, lateral view.

Fig. 16. Egg containing embryo.

Fig. 17. Median sagittal section of the 'female' worm, showing ventral groove and positions of the ovary and yolk glands.

in the Didymozoinae. The present investigation into the anatomy of *K. filicollis* has shown that the sexes are not entirely separate in members of the subfamily Kollikeriinae and therefore it cannot be distinguished from the Didymozoinae.

Yamaguti (1953) listed and described briefly twenty-two genera for the family Didymozoidae. His key to the genera, however, is unsatisfactory as it does not distinguish clearly between them, for example *Tricharchen* Poche, 1926, *Coeliotrema* Yamaguti, 1938, and *Köllikeria* Cobbold, 1860. Very little is known about *Tricharchen okenii* (Kölliker, 1849), the only species of this genus, and as it was found by Kölliker on the same host and in the same habitat as *Köllikeria filicollis*, that is *Brama raii*, it must be regarded as a genus and species *inquirenda*. Yamaguti's diagnoses for the genera *Köllikeria* and *Coeliotrema* are almost identical, but according to him they differ in their host distribution, in the absence of an acetabulum in *Coeliotrema* and in the presence of rudimentary female genitalia in the males of this latter genus. Yamaguti (1953), however, made the following comment on the acetabulum of the genus *Köllikeria*: 'this organ, being subject to degeneration is not of more than specific significance in the family under consideration.' He also placed *Wedlia orientalis*, a species which possesses rudimentary female genitalia in the 'male', in the genus *Köllikeria*. The only character left, therefore, to distinguish his genus *Coeliotrema* from *Köllikeria* is host distribution and this is not accepted by the writer as being of generic importance.

Yamaguti (1953) placed five species in the genus *Köllikeria*, namely *K. filicollis* (Rud., 1819), Cobb., 1860, *K. bipartita* (Wedl., 1855), *K. globosa* Ishii, 1935, *K. orientalis* (Yamaguti, 1934) and *K. reniformis* Ishii, 1935. He stated, Yamaguti (1934), that *K. orientalis* resembles *K. bipartita* so closely that they can only be distinguished by the size of the eggs. *K. filicollis* appears to be more closely related to *K. orientalis* than it is to the other four species in the genus.

IX. DIAGNOSTIC FEATURES OF *KÖLLIKERIA FILICOLLIS*

Cyst-dwelling member of the Didymozoidae Poche, 1907. Sexes not entirely separate but the functional 'males' and 'females' are sexually dimorphic.

'Male' greyish white, filiform, 13·50–17·00 mm. long and 0·41–0·78 mm. broad; ventral sucker present; alimentary canal extends to posterior end of body, the caeca undulate; testis single, 11–12 mm. in length a short distance behind ventral sucker.

'Female' orange yellow, divided into fore- and hind-body; shape of fore-body like anterior half of 'male'; hind-body swollen and reniform; ventral sucker present in fore-body; alimentary canal does not extend far into the hind-body; walls of the caeca straight with club-shaped distal ends; distal end of unbranched ovary almost club-shaped; yolk gland single unbranched; uterus thick-walled, muscular in anterior region of hind-body; eggs 0·021 mm. long and 0·016 mm. broad. Habitat: 'males' and 'females' found in orange-yellow cyst attached to the epithelium beneath the operculum near the posterior gills of *Brama raii* Bloch.

X. SUMMARY

The anatomy of *Köllikeria filicollis* (Rudolphi, 1819), Cobbold, 1860 (Trematoda: Digenea) is described in detail.

An account is given of the integument and musculature of the worm and the structure of the cyst in which it is found, followed by a discussion of the body wall in other digenetic trematodes. The alimentary canal, excretory system, nervous system and genitalia are also described.

The study shows that the sexes are not entirely separate in all members of the genus *Köllikeria* as hitherto believed.

The systematic position of *K. filicollis* is discussed and a specific diagnosis is given for the species.

The writer is deeply indebted to Dr Gwendolen Rees for her valuable guidance in the preparation of the above work, to Professor T. A. Stephenson, F.R.S., for his interest and encouragement, to Mr S. Prudhoe of the British Museum for his advice on many taxonomic problems on helminth parasites and for allowing me to examine specimens and literature, to Mr H. Atkinson, Managing Director, J. Marr and Son Ltd., Fleetwood, for making arrangements for me to sail on M.T. *Irvana* and to the Department of Scientific and Industrial Research who made the investigation possible by giving generous financial aid.

KEY TO LETTERING

b.	brain	*oe.*	oesophagus
b.m.	basement membrane	*oe.g.*	oesophageal glands
c.c.	central chamber	*om.*	operculum
c.m.l.m.	circular and longitudinal muscles of body wall	*o.s.*	oral sucker
		ov.	ovary
cu.	cuticle	*ovd.*	oviduct
cy.w.	cyst wall	*ph.*	pharynx
d.a.n.	dorsal anterior nerve	*r.s.*	receptaculum seminis
e.	egg	*s.*	crown of spines
em.	embryo	*s.c.g.*	subcuticular gland cell
e.p.	excretory pore	*sp.*	spermatozoa
e.v.	excretory vessel	*s.s.c.g.*	secretion from subcuticular gland cell forming inner wall of cyst
g.	ganglion		
g.a.	genital atrium	*t.*	testis
f.b.	fore-body	*t.c.*	transverse commissure
h.b.	hind-body	*ut.*	uterus
h.t.	host tissue	*ut.m.*	muscular region of uterus
i.	intestine	*v.a.n.*	ventral anterior nerve
l.a.n.	lateral anterior nerve	*v.d.*	vas deferens
l.n.	lateral nerve	*v.g.*	ventral groove
ly.	lymphocyte	*v.g.c.*	cavity of ventral groove
m.	mouth	*v.g.w.*	extent of lateral wall of ventral groove
me.	melanophore		
m.g.	Mehlis's gland	*v.n.*	ventral nerve
m.w.	'male' worm	*v.s.*	ventral sucker
myo.	myoblast	*y.*	yolk gland
n.c.t.	nucleus of connective tissue	*y.c.*	yolk cell
n.s.c.g.	nucleus of subcuticular gland cell in cuticular pore	*y.d.*	yolk duct

REFERENCES

BAYLIS, H. A. (1938). On two species of the trematode genus *Didymozoon* from the mackerel. *J. Mar. Biol. Ass. U.K.* **32**, 485–92.

DAWES, B. (1946). *The Trematoda*, 644 pp. Cambridge University Press.

DOLLFUS, R. PH. (1926). Sur l'état actuel de la classification des Didymozoonidae Monticelli, 1888 (Didymozoidae Poche, 1907). *Ann. Parasit. hum. comp.* **4**, 148–61.

HUNTER, G. W. & HAMILTON, J. M. (1941). Studies on host-parasite reactions to larval parasites. IV. The cyst of *Uvulifer ambloplitis* (Hughes). *Trans. Amer. Micr. Soc.* **60**, 498–507.

ISHII, N. (1935). Notes on the family Didymozoonidae (Monticelli, 1888). *Japan. J. Zool.* **6**, 279–335.

NIGRELLI, R. F. (1939). *Didymocystis coatesi*, a new monostome from the eye muscles of the Wahoo, *Acanthocybium solandri* (C.V.). *Trans. Amer. Micr. Soc.* **58**, 170–8.

ODLAUG, T. O. (1948). The finer structure of the body wall and parenchyma of three species of digenetic trematodes. *Trans. Amer. Micr. Soc.* **67**, 236–53.

PRATT, H. S. (1909). The cuticula and sub-cuticula of trematodes and cestodes. *Amer. Naturalist*, **43**, 705–20.

STEPHENSON, W. (1947). Physiological and histochemical observations on the adult liver fluke, *Fasciola hepatica* L. III. Egg shell formation. *Parasitology*, **38**, 128–39.

STUNKARD, H. W. (1946). Interrelationships and taxonomy of the digenetic trematodes. *Biol. Rev.* **21**, 148–58.

WAGENER, G. R. (1858). Enthelminthica. No. 6. Ueber *Distoma campanula* (*Gasterostoma frimbriatum* Siebold) Duj. und *Monostoma bipartitum* Wedl. *Arch. Naturgesch.* 24. Jahrg., **1**, 250–6.

YAMAGUTI, S. (1934). Studies on helminth fauna of Japan. Part 2. Trematodes of fishes, 1. *Japan. J. Zool.* **5**, 249–541.

YAMAGUTI, S. (1938). Studies on the helminth fauna of Japan. Part 24. Trematodes of fishes. *Japan. J. Zool.* **8**, 15–74.

YAMAGUTI, S. (1953). *Systema helminthum. Part* 1. *Digenetic trematodes of fishes*, 405 pp. Tokyo: Maruzen Co.

YOSUFZAI, H. K. (1953). Shell gland and egg shell formation in *Fasciola hepatica* L. *Parasitology*, **43**, 88–94.

(*MS. received for publication* 2. XII. 1957.—Ed.)

THE ANATOMY OF *PHYLLOBOTHRIUM SINUOSICEPS* SP.NOV. (CESTODA: TETRAPHYLLIDEA) FROM *HEXANCHUS GRISEUS* (GMELIN) THE SIX GILLED SHARK

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(With Plate II containing Figs. 1–6, and Figs. 1–13 in the Text)

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I. INTRODUCTION

Many workers, including Beneden (1849, 1850*a*, *b*, 1858–61), Braun (1894–1900), Beauchamp (1905), Poche (1925), Southwell (1925, 1930), Woodland (1927), Perrenoud (1931), Joyeux & Baer (1936), Wardle & McLeod (1952) and Riser (1955), have given accounts of cestodes of the order Tetraphyllidea. An examination of this literature and other papers shows nothing which agrees with the species described below.

The description is based upon a study of fifteen entire strobilae and fifty free segments found in the spiral valve of four specimens of *Hexanchus griseus* trawled from a depth of about 150 fathoms by the Milford Haven motor-vessel *Milford Duke* at latitude 54° 0′ N. and longitude 11° 15′ W. The parasites were fixed in 5% formaldehyde. Temporary mounts of fixed specimens were examined under a coverslip in water, lactophenol or a solution of glycerine in alcohol. For permanent whole mounts Coelestin blue or Ehrlich's haematoxylin were used as stains, while serial sections cut in three planes, transverse, horizontal and sagittal were stained with Mallory's triple stain or with Ehrlich's haematoxylin and eosin or Orange G.

It was thought advisable to compare *Phyllobothrium sinuosiceps* with *P. dohrnii* described in detail by Rees (1946), as the majority of previous accounts of other members of the genus *Phyllobothrium* are inadequate.

II. EXTERNAL FEATURES

(*a*) *Scolex*

The scolex, in living specimens attached to the mucosa of the intestine, covers a circular area up to 12 mm. in diameter. On fixation in formaldehyde it may remain attached (Pl. II, fig. 1). contracting together with the intestine to an area of only 6 mm. or less. In fixed specimens, detached from the mucosa before fixation,

Table 1. *Comparison of* Phyllobothrium sinuosiceps *sp.nov. with two other species of the genus*

Species	*Phyllobothrium sinuosiceps* sp.nov.	*Phyllobothrium lactuca* Beneden	*Phyllobothrium radioductum* Kay
Type host	*Hexanchus griseus* (Gmelin)	*Mustelus vulgaris*, Day	*Raja binoculata* (Girard)
Scolex	Variable in size. Not divided into four distinct bothridia. Not armed with minute spines	Variable in size. Apparently divided into four much folded bothridia. Not armed with minute spines	Variable in size. Apparently divided into four much folded bothridia. Armed with minute spines
Suckers	Weakly developed 0·25 mm. in diameter	Weakly developed 0·208 mm. in diameter	Weakly developed. Diameter not known
Average length of strobila in mature specimen	200 mm.	200 mm.	39 mm.
Cuticle	Scaly	Apparently not scaly	Scaly
Excretory system of scolex	Simple	Details not known	Complicated and much branched
No. of testes	700–800	Approx. 250	Approx. 250
Cirrus sac	1·95–2·4 mm. in length, 0·15–0·30 mm. in breadth	0·38–0·57 mm. in length, 0·15–0·16 mm. in breadth	Not known
Vagina	Thin walled	Possesses a thick muscular wall	Thin walled and possessing a well-developed terminal vaginal sphincter
Uterine pore	Absent	Present	Absent

the scolex is almost spherical, varying in diameter from 3·20 to 6·00 mm. It is variable in shape and no two are identical, but in general terms the scolex is provided with a series of complicated folds (Text-figs. 2, 8). The folds may be differently arranged, but usually there are two to four folds encircling the scolex either completely or incompletely, the interruptions in the folds making it very difficult to follow their complete course. These, which will be referred to as the

main folds, are doubled, trebled or even subdivided along their edges (Pl. II, figs. 1, 2; Text-fig. 8). In addition, the edges of the main folds and those of their subdivisions are frilled. The posterior folds tend to be pendulous and therefore overlap the anterior part of the strobila (Text-fig. 8). There are four small suckers (Text-figs. 2, 8), varying slightly in position, situated on the folds at the apex of the scolex. Each sucker measures about 0·25 mm. in diameter; there is no myzorhynchus.

(*b*) *Strobila and free segments*

In the living state *Phyllobothrium sinuosiceps* may attain a length of 350 mm. but on fixation it may contract to 200 mm. The breadth of the strobila is 1·50 mm. immediately behind the scolex, compared with 5 mm. at the posterior end; the strobila measures 0·70 mm. in depth anteriorly and 1·80 mm. posteriorly. The neck (Text-figs. 1, 2) appears to occupy a quarter to a third of the length of the strobila. The segments which follow vary in shape, being usually broader than long (Text-figs. 3–6), the posterior segments may attain a length of 5·20 mm. which is slightly greater than the breadth. *P. sinuosiceps* is euapolytic, that is, growth and egg production continue after the segments have become detached. Detached living segments contract and expand continuously and rhythmically. Their shape, therefore, after fixation is variable, but in contrast to the segments of the strobila their length, which is 5·00–12·60 mm., is always considerably greater than the breadth (Text-fig. 7). The genital atrium is lateral, irregularly alternate and usually slightly in front of the middle of the segment.

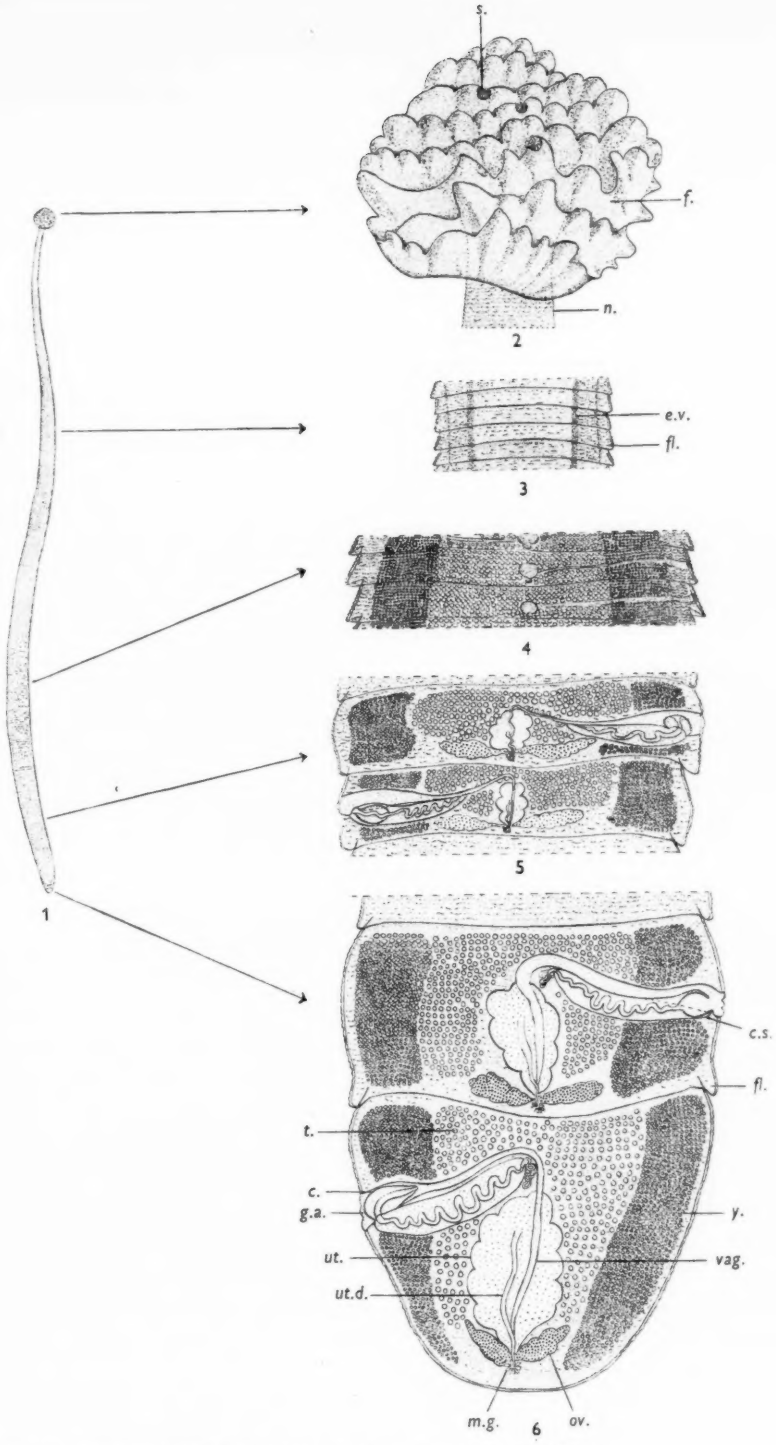
III. INTEGUMENT AND MUSCULATURE

The musculature is very much like that of *P. dohrnii*.

(*a*) *Strobila*

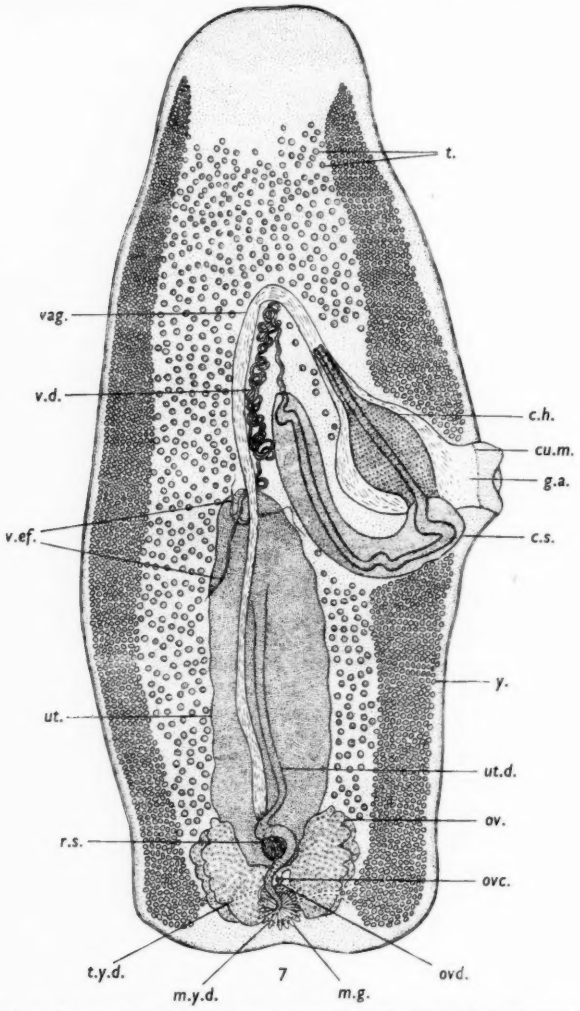
The cuticle, which is scaly (Pl. II, figs. 5, 6; Text-fig. 8) rests on a basement membrane, below which lies the superficial musculature consisting of outer circular and inner longitudinal layers of muscle fibres. The circular muscle fibres, which are delicate, are equally developed throughout the length of the strobila. The longitudinal muscles are strongest in the neck region where they form a layer 0·045 mm. deep and comprise eight fibres. They are not so prominent towards the posterior end of the strobila, the layer there being difficult to detect.

The deep longitudinal muscle layer in the neck region forms a continuous ring 0·25 mm. deep around the medulla (Pl. II, fig. 1; Text-fig. 8). This layer is distinct, separated from the superficial musculature and consists of individual fibres which are not arranged in bundles, each fibre measuring 0·005 mm. in diameter. Towards the posterior end of the strobila and in the free segments the deep longitudinal muscle fibres are, however, arranged in bundles and the layer itself merges into the superficial musculature. The foregoing variations in the musculature within a single species are of importance as there is some controversy in the literature regarding the arrangement of the muscles in the families Phyllobothriidae and the Proteocephalidae. Woodland (1927) was of the opinion that in the Phyllo-



Text-fig. 1. *Phyllobothrium sinuosiceps* sp. nov., complete strobila.
Text-fig. 2. Anterior view.
Text-figs. 3–6. Segments from different regions of the same complete strobila, dorsal view.

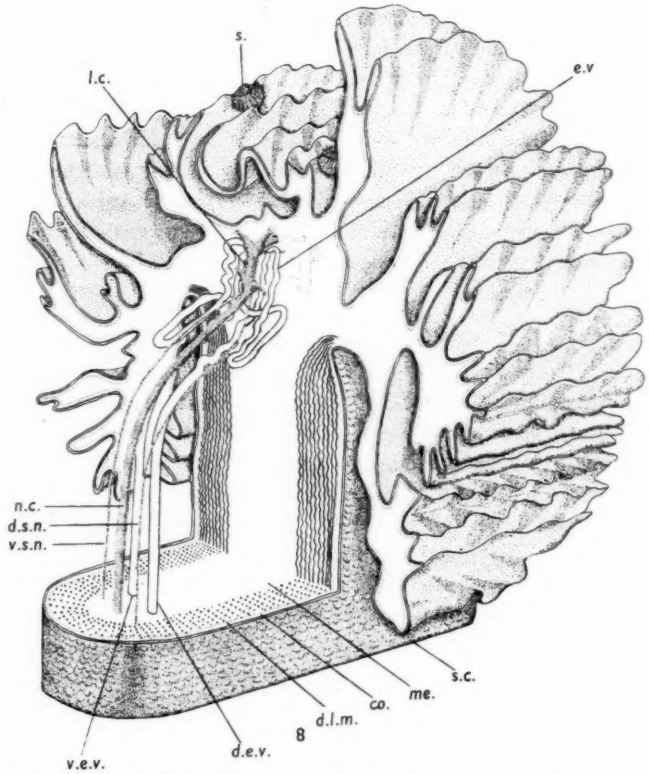
bothriidae in contrast to the Proteocephalidae 'the parenchyma is not divided by an internal layer of longitudinal muscle bundles into cortex and medulla, the longitudinal musculature being restricted to an undivided peripheral zone of



Text-fig. 7. *Phyllobothrium sinuosiceps* sp. nov., free segment showing male and female genitalia, dorsal view.

fibres and bundles of fibres lying immediately internal to the thin circular muscle layer underlying the cuticle and more or less co-extensive with the subcuticula'. Southwell (1929) did not agree with Woodland, stating that striking variations may occur in the musculature of different regions of an individual of the same species and therefore he came to the conclusion that the musculature is of no taxonomic value. The variations recorded for *Phyllobothrium sinuosiceps* confirm

Southwell's view that variations do occur within a single species, but Woodland (1927) may be justified in stating that the arrangement of the musculature is of some value to the taxonomist for the following reason. Rees (1946) has shown clearly that in *P. dohrnii* the shape of the deep longitudinal muscle band in the anterior region of the strobila is characteristic for this species and can be correlated



Text-fig. 8. *Phyllobothrium sinuosiceps* sp.nov., stereograph of scolex showing folding of its surface, nervous, excretory and muscular systems.

with an overlapping of the segments. An overlapping of the segments also occurs in *P. sinuosiceps*, but there is no correlated modification of the deep longitudinal musculature. Differences of this nature, therefore, may prove to be of value in the identification of some members of the Phyllobothriidae.

Radial and dorso-ventral muscles are present and are most easily seen in the first half of the strobila, although they are weakly developed throughout its length. The detail of these muscles is the same as in *P. dohrnii*.

(*b*) *Scolex*

The arrangement of the musculature in the scolex resembles very closely that of *P. dohrnii*. A detailed description of this system, therefore, will not be given

here. The superficial muscles of the strobila continue into the scolex where they become more extensive, forming a complicated network underneath the basement membrane. In serial sections of the scolex this elaborate network of fibres has the appearance of sections through a very broad shallow sucker (Pl. II, figs. 3, 4), as is also the case in sections of the bothridia of *P. dohrnii*.

The deep longitudinal musculature is almost indentical with that of *P. dohrnii*. At the base of the scolex, the muscle fibres are arranged in a uniform ring, but on entering the scolex they spread out, extending into the folds (Pl. II, figs. 2, 3) where they become inserted into the basement membrane anywhere in the wall of a fold. In the scolex there is, in addition, a system of deep oblique muscle bands which, by crossing one another, demarcate a rectangular area (Pl. II, fig. 3), enclosing the excretory vessel and parts of the nervous system. The apical suckers are composed mainly of radial muscles and possess two extrinsic muscles inserted into their lateral margins as in those of *P. dorhnii*. They are demarcated from the underlying parenchyma, but are not as strongly developed as in *P. dohrnii*.

IV. NERVOUS SYSTEM

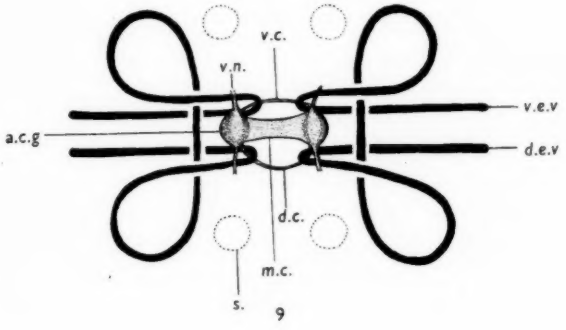
The 'brain' (Pl. II, fig. 2; Text-figs. 9–10) consists of right and left anterior and posterior cerebral ganglia situated in the scolex. The anterior ganglia (Text-fig. 9) are ill defined and measure 0·075 mm. in diameter. They are connected with one another by a slightly curved median commissure which is 0·400 mm. long and 0·090 mm. broad. The ganglia are also connected by a much more delicate ring commissure situated slightly behind the median commissure. The posterior ganglia (Pl. II, fig. 2; Text-fig. 10) which are a short distance behind the anterior ganglia are ill defined and are 0·075 mm. in diameter. A lateral longitudinal connective, 0·230 mm. long and 0·060 mm. in diameter, joins each anterior ganglion to the posterior ganglion that lies behind it. From each of the anterior ganglia (Text-fig. 9) arises a dorsal and a ventral nerve, the course taken by these four nerves indicating that they supply the four suckers of the scolex. From each of the posterior ganglia (Text-fig. 9) arises a dorsal and a ventral nerve to the scolex as well as three other nerves which, as will be seen below, supply the strobila.

The 'brain' of *P. sinuosiceps* is extremely interesting when compared with that of *P. dohrnii* which consists of two dorsal and two ventral ganglia, each ganglion giving off two nerves. The ganglia are connected by a transverse and a ring commissure. If one imagined that the lateral longitudinal connective of the brain of *P. sinuosiceps* was so diminished that the anterior and posterior ganglia fused, then the brain would be almost identical with that of *P. dohrnii*. It is possible that a partial fusion of the ganglia may have occurred in the evolution of the brain in the Tetraphyllidea as there is slight evidence from its host distribution and some other anatomical features that *P. sinuosiceps* may be a primitive member of the order.

The nervous system in the strobila of *P. sinuosiceps* consists of three nerves on each side of the body (Text-figs. 8, 10). They are the median nerve cord and the dorsal and ventral subsidiary nerves, all of which extend as far as the posterior extremity.

V. EXCRETORY SYSTEM

The excretory system of *P. sinuosiceps* consists, in the main, of dorsal and ventral longitudinal vessels in the strobila (Text-fig. 8) which are connected in the scolex to form loops on either side (Text-fig. 9).



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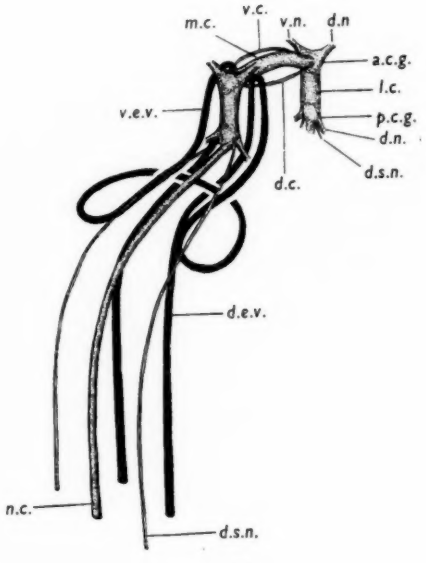

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Text-fig. 9. *Phyllobothrium sinuosiceps* sp.nov., brain and excretory system, anterior view.
Text-fig. 10. Brain and excretory system, lateral view.

The dorsal and ventral longitudinal vessels run throughout the length of the strobila, the ventral vessels being connected at the posterior margin of each segment by a transverse vessel. The four vessels are of uniform diameter in the neck region (Pl. II, fig. 1; Text-fig. 8), but more posteriorly the ventral vessels increase while there is a decrease in size of the dorsal vessels.

The general arrangement of the excretory vessels in the scolex is like that of *P. dohrnii*. The dorsal and ventral vessels of each side of the strobila extend into the scolex and take a similar course (Text-figs. 8–10). Those of one side only will be described. The dorsal and ventral vessels converge somewhat on entering the scolex and extend as the ascending vessels as far as the median commissure of the brain. From here both vessels, which lie one on each side of the median commissure, loop outwards over the ring commissure passing back as the descending vessels which run more or less parallel to the ascending vessels. The dorsal and ventral vessels on each side continue backwards as far as the posterior margin of the scolex; here they diverge slightly, each one looping back towards the centre of the scolex, the two vessels meeting to become continuous with one another. The looping of the excretory vessels in the scolex is not so extensive as that of other members of the genus *Phyllobothrium*, the vessels appearing to be more elaborate in those species which possess well-developed bothridia. The simpler arrangement of the excretory system of *P. sinuosiceps*, together with the simpler arrangement of the brain, suggests that it may represent a stage in the evolution of a scolex with four bothridia rather than a fusion of four bothridia.

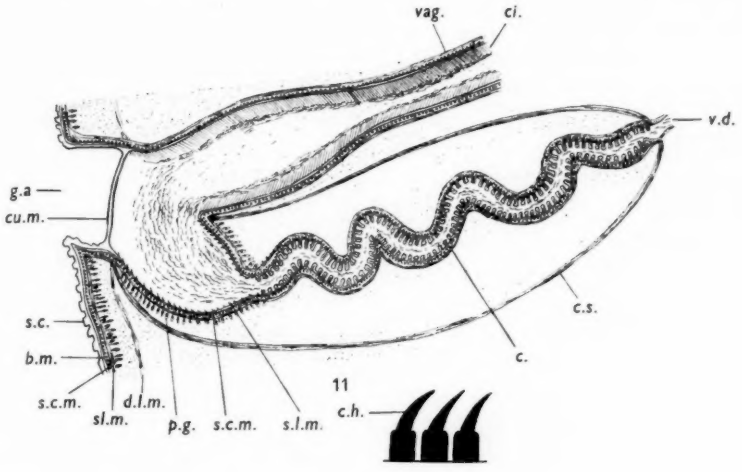
VI. GENITALIA

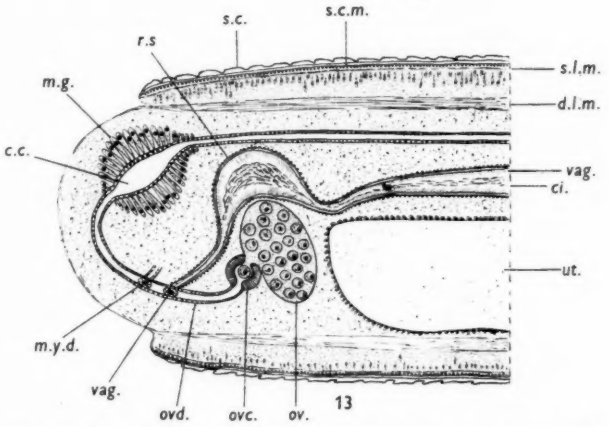
The male and female genitalia in *P. sinuosiceps* ripen together in the same region of the strobila, there being no evidence of protandry. Rudiments of both systems first appear in the middle region and mature progressively from there to the posterior end (Text-figs. 4–7). The genital pore is irregularly alternate, the genital atrium measuring 0·3 mm. deep and 0·30 mm. broad. A very thin cuticular membrane continuous with the cuticle of the body wall extends over the genital pore, permanently covering the aperture (Text-figs. 7, 11). The writer is not aware of a similar membrane existing in other members of the Tetraphyllidea. The possible significance of this will be discussed later.

(*a*) *Male genitalia*

There are between 700 and 800 testes in each segment (Text-fig. 7). In the anterior half they occupy the central region while more posteriorly they are lateral, being interrupted on one side by the vagina and cirrus pouch. The testes are three to four layers deep, spherical in shape and measure 0·105 mm. in diameter. Vasa efferentia arise from the testes and converge towards the centre of the segment where they unite in groups to form three main vessels which in turn unite to form the very much coiled vas deferens which extends forwards. The muscular cirrus sac, which is obliquely placed to the genital atrium, lies postero-dorsal to the vagina and is 1·95–2·40 mm. long and 0·15–0·30 mm. broad. The vas deferens enters the cirrus sac within which it becomes continuous with the muscular cirrus (Text-fig. 11). The cirrus in some segments is invaginated (Text-fig. 11), its lining being folded like a bellows, while in others it is evaginated and can be seen to extend into the vagina of the same segment (Text-fig. 7) for a distance varying from 2·00 to 5·25 mm. In some specimens (Pl. II, fig. 5) the evaginated cirrus

extends along the whole length of the vagina as far as the level of the ovary. Only in two segments out of fifty was the cirrus found protruding to the exterior through the genital atrium, having ruptured the cuticular covering of the genital pore.





Text-fig. 11. *Phyllobothrium sinuosiceps* sp.nov., horizontal section through part of free segment, showing cirrus sac and vagina.

Text-fig. 12. Cirrus hooks.

Text-fig. 13. Median sagittal section through posterior region of free segment, showing parts of female genitalia.

This condition, however, is abnormal for *P. sinuosiceps*, which point will be discussed later. The mechanism by which the cirrus is evaginated and invaginated depends on the arrangement of its muscles and on those of the cirrus sac. Longitudinal muscles derived from the body wall are predominant in the wall of the cirrus sac (Text-fig. 11) and, as they contract, pressure is exerted within the sac and therefore on the cirrus. If the cirrus is filled with spermatozoa, as it is in all

the specimens examined, its walls cannot collapse to compensate for the pressure exerted upon it, and even if it did collapse pressure would be created within the vagina as the genital pore is blocked with a cuticular membrane. To avoid damage, therefore, the only available mechanism seems to be for the circular muscles of the cirrus to relax allowing for it to roll outwards and into the vagina. The basal portion of the evaginated cirrus (Text-fig. 7) is swollen, being almost spherical in shape and contains prostate gland cells. The terminal end of the vagina is permanently dilated, presumably to receive the swollen basal portion of the evaginated cirrus. The cirrus is armed with hooks (Text-fig. 12), 0·014 mm. long, each hook having the spine sharply demarcated by a shoulder from a swollen cylindrical base. The hooks presumably have the function of holding the evaginated cirrus within the vagina.

(*b*) *Female genitalia*

The bilobed ovary which is X-shaped in transverse section is situated at the posterior end of the segment (Text-fig. 7). The oviduct arises as an ovicapt (Text-fig. 13) on the ventral surface of the ovary and continues posteriorly. It receives first the vagina then the median yolk duct before looping antero-dorsally. In the course of the loop the oviduct becomes dilated to form the central chamber which is surrounded by Mehlis's glands (Pl. II, fig. 6; Text-fig. 13). The oviduct emerges from the Mehlis's gland as the slightly sinuous uterine duct (Text-figs. 7, 13), which continues anteriorly in the mid-dorsal line. It opens into the dorsal side of the sac-like uterus near the level of the genital atrium (Text-fig. 7). As the uterus fills with eggs it becomes more extensive and its surface is lobed. There is some controversy in the literature concerning the form of the uterus in the gravid segments of some members of the Phyllobothriidae, for instance *P. lactuca* Beneden, 1850*b*. According to Beneden (1850*b*) and Southwell (1925), only the free segments are gravid. Yoshida (1917) and Woodland (1927), however, noted that the terminal proglottides of *P. lactuca* do not become free and that they contain uteri distended with eggs which are discharged through median uterine pores. Linton (1897) and Southwell (1930) stated that in most species of the Phyllobothriidae examined by them no uterine pores were discovered. Uterine pores are absent in *P. sinuosiceps* and the uteri of both the terminal segments of the strobila and those of the free segments contain eggs. The vagina, as already mentioned, has a dilated distal end, a feature of the anatomy of the Phyllobothriidae referred to by Southwell (1930) as the vulva. From the 'vulva' the vagina takes an anterior course towards the middle line becoming slowly dorsal in position before turning sharply backwards. It runs posteriorly in the middle line between the uterus and the uterine duct as far as the dorsal side of the ovary where it becomes enlarged to form a receptaculum seminis. The latter narrows posteriorly before turning towards the ventral side to open into the oviduct (Text-fig. 13). The length of the vagina varies from 3·45 to 10·50 mm. depending on the degree of development of the segment. The lining of the vagina is ciliated throughout its length. According to Pintner (1934*b*) the cilia which line the lumen of the vagina in members of the Phyllobothriidae are non-motile, functioning as a non-return valve for the sperma-

tozoa. The cilia in *P. sinuosiceps*, however, may be motile and their function may be to aid the passage of spermatozoa towards the receptaculum seminis. The nature and disposition of the cilia (Text-fig. 11), in relation to masses of spermatozoa as seen from serial sections, supports this view. The yolk glands (Text-fig. 7) are situated laterally, forming a broad band on each side of the segment extending along its whole length on the inner side of the nerve cords enveloping the excretory vessels and the testes.

Some features of the male and female genitalia are interesting and will be discussed in relation to fertilization. According to Wardle & McLeod (1952) very little is known regarding copulation in the Cestoda. They state: 'In spite of the general belief that an extruded cirrus can be inserted readily into the vagina of a proglottid in another part of the strobila, few observations exist to support it. Some authorities have thrown doubt upon the supposition. There is a strong suspicion that in the majority of tapeworms there is a single emission of semen into the fluid environment and an active migration of sperm cells from the environment into the vagina.'

According to Hyman (1951) Wolfhügel is of the opinion that the cirrus is armed with large hooks in the Acoleidae of the Cyclophyllidea because a vagina is lacking and hypodermic impregnation takes its place as a method for fertilization. It is thought that none of these methods of fertilization applies in the case of *P. sinuosiceps*, the observed anatomical details of the genitalia suggesting that self fertilization of individual segments occurs in this species. Self fertilization would appear to be essential to the species as free segments, after their liberation from the strobila, continue to grow, producing large numbers of eggs. The cuticular membrane covering the genital pore, the structure and mode of function of the cirrus and cirrus hooks, the ciliated vagina and its 'vulva' may, therefore, be adaptations to ensure self fertilization and are the result of specialization in a primitive species. The function of the cuticular membrane (Text-figs. 7, 11) may be to prevent the cirrus from evaginating through the genital pore. The membrane, however, might be perforated by the cirrus and cross fertilization might be achieved but, as has already been stated, only in two segments out of fifty was the cirrus found protruded. In all the others it was either evaginated into the vagina of its own segment or remained invaginated within the cirrus pouch. The cirrus hooks suggest that hypodermic impregnation of another segment might occur, but the cirrus hooks which are more concentrated at the base of the evaginated cirrus are so disposed that the cirrus could not be withdrawn again, even for a distance of a few millimetres, without some damage to the surrounding tissues. No such damaged segment has been found. Cross-fertilization could possibly take place if the genital atria of two segments of the strobila, two free segments or a free segment and a segment of the strobila came into direct contact, but the cirrus of one segment has not been seen within the vagina of another. Again, the relative position, size and shape of the 'vulva' in relation to the spherical swelling at the base of the evaginated cirrus suggests that its function may be to accommodate the cirrus of its own segment. The possibility that the cilia may help to bring about self fertilization cannot be ruled out because serial sections of the cirrus and vagina show that the cilia may

aid the transportation of masses of spermatozoa towards the receptaculum seminis even when the cirrus is invaginated. Lastly there is no need for cross-fertilization in *P. sinuosiceps* because it is not protandric. When protandry does occur in the Cestoda, as is usually the case, it is generally agreed that young male segments fertilize the more posterior and older female segments. On the other hand, cross-fertilization would be difficult to achieve in this instance as the species is euapolytic and the chances of free segments meeting in the spiral valve are probably rare, especially if only a few segments are present.

VII. TAXONOMY OF *PHYLLOBOTHRIUM SINUOSICEPS* SP.NOV.

Phyllobothrium sinuosiceps has been tentatively placed in the genus *Phyllobothrium* Braun, 1900, of the Phyllobothriidae, because it resembles many other members of the genus including *P. lactuca* Beneden, 1850, which is the genotype of the family. Unfortunately, there is no detailed description of *P. lactuca*. The original description by van Beneden and those by subsequent authors, namely Yoshida (1917), Southwell (1925), Woodland (1927), Borcea (1934), Joyeux & Baer (1936*d*) and Riser (1955) are very brief and do not agree with one another. As a detailed description of the genotype is not available a large number of species have been placed in the genus *Phyllobothrium* because they cannot be placed in any of the other ten genera of the family. It is clear from all previous definitions of the genus by Beneden (1849, 1850*b*), Braun (1894–1900), Linton (1924), and Southwell (1925) that the external form of the scolex was regarded as an important taxonomic character and one cannot ignore this completely at present as little or nothing is known regarding the rest of the anatomy of many of the species. The result is that over thirty species have been assigned to the genus *Phyllobothrium* and the majority of these are inadequately described. *P. sinuosiceps* may have to be placed in a new genus when a complete description of *P. lactuca* has been made, but at present there is no alternative but to include it provisionally in the genus *Phyllobothrium*. The scolex of *P. sinuosiceps* agrees with that of *P. lactuca* in size, in the presence of four apical suckers and in being very much folded 'like the leaves of a lettuce'; it differs only in that it does not bear four well-defined bothridia. As, however, many figures of *P. lactuca* indicate clearly that it may not bear four distinct bothridia, this difference cannot be regarded as a generic character. In addition, it should be mentioned that the scolices of many other species, including *P. crispatissimum* Monticelli, 1889, *P. radioductum* Kay, 1942, *P. thridax* Zschokke, 1888, and *P. unilaterale* Beneden, 1850, are also characterized by the same kind of scolex. It has been an extremely difficult task to separate *P. sinuosiceps* from any which is closely allied to it, but having examined all the information that was available on previously described species, and Woodland's (1927) material of *P. lactuca* at the British Museum, it has been concluded that it is related to *P. lactuca* and *P. radioductum*. In our present state of knowledge the three species can be distinguished by the characters shown in the accompanying table. It can be seen that *P. sinuosiceps* differs from *P. lactuca* in the host species, in the absence of well-defined bothridia, in the nature of the cuticle, in the larger number of testes, in the size of the cirrus sac

relative to the segment in which it is contained, in the nature of the vagina and in the absence of uterine pores. *P. sinuosiceps* differs from *P. radioductum* in the host species, in its larger size, in the absence of well-defined bothridia armed with minute spines, in its simple, compared with a complicated, excretory system, in the larger number of testes and in the absence of a vaginal sphincter.

One aspect of the evolution of the Tetraphyllidea becomes apparent as a result of this study and will be discussed briefly here. According to Baer (1952) the most primitive tapeworms are found in primitive vertebrate hosts which are undoubtedly the fishes. The discovery of *P. sinuosiceps*, therefore, is interesting as it was found in *Hexanchus griseus*, a member of the Notidanoidea, the most primitive order of existing sharks. As several specimens were collected from two sharks accidental infestation can be ruled out, and it is reasonable to assume that the species may be a primitive member of the Tetraphyllidea. The simple arrangement of the nervous and excretory systems and the specialized features of the male and female genitalia support the view that *P. sinuosiceps* may be a primitive species. In addition to these the presence of two dorsal and two ventral, but weakly developed suckers, and the resemblance of the musculature of the scolex to that of *P. dohrnii*, suggest that the types with four prominent bothridia, each bothridium possessing a well-marked sucker may have arisen from a form like *P. sinuosiceps*.

VIII. DIAGNOSTIC FEATURES OF *PHYLLOBOTHRIUM SINUOSICEPS* SP.NOV.

Strobila up to 350 mm. long, cuticle scaly; attached segments craspedote, broader than long; euapolytic, free segments longer than broad; scolex almost spherical in shape, 3·20–6 mm. in diameter, possessing four apical suckers not clearly divided into four distinct bothridia but bearing two to four complicated folds encircling the scolex, the folds are subdivided and their edges frilly; myzorhynchus absent; arrangement of the deep longitudinal muscles not correlated with the overlapping of the segments; excretory and nervous systems of the scolex simple; genital atrium covered permanently by a cuticular membrane; testes between 700 and 800; cirrus pouch 1·95–2·40 mm. long, 0·15–0·30 mm. broad; evaginated cirrus 5·25 mm. long, swollen basally, armed with small hooks, 0·014 mm. in length, with sharp points and swollen cylindrical bases; vagina thin walled, dilated proximally to form a 'vulva'; uterine pore absent. Host: *Hexanchus griseus* Gmelin. Location: spiral valve.

IX. SUMMARY

The external features, the excretory, nervous and genital systems of *Phyllobothrium sinuosiceps* sp.nov. (Cestoda: Tetraphyllidea) from *Hexanchus griseus* are described in detail, particular attention being given to the genitalia.

The species is compared with *Phyllobothrium dohrnii*, a well-known cestode.

The systematic position of *P. sinuosiceps* is discussed and the differences between *P. sinuosiceps*, *P. lactuca* and *P. radioductum* are tabulated.

The simple arrangement of the nervous and excretory systems, the specialized features of the genitalia and host distribution suggest that *P. sinuosiceps* may be one of the most primitive species belonging to the family Phyllobothriidae.

The writer is deeply indebted to Dr Gwendolen Rees for her invaluable guidance in the preparation of the above work, to Professor T. A. Stephenson, F.R.S., for his interest and encouragement, to Mr S. Prudhoe of the British Museum for his advice on many taxonomic problems on helminth parasites and for allowing me to examine specimens and literature, to Mr H. Rossant, O.B.E., Secretary, Trawler Owners Association Ltd., Milford Haven, for making arrangements for me to sail on motor vessel *Milford Duke*, and to the Department of Scientific and Industrial Research who made the investigation possible by giving generous financial aid.

KEY TO LETTERING OF TEXT-FIGURES

a.c.g.	anterior cerebral ganglion	*n.c.*	nerve cord
b.m.	basement membrane	*o.d.m.*	oblique deep muscles of scolex
c.	cirrus	*ov.*	ovary
c.c.	central chamber of Mehlis's gland	*ovc.*	ovicapt
c.h.	cirrus hooks	*ovd.*	oviduct
ci.	cilia	*p.c.g.*	posterior cerebral ganglion
co.	cortex	*p.g.*	prostatic gland cell
c.s.	cirrus sac	*r.s.*	receptaculum seminis
cu.m.	cuticular membrane	*s.*	sucker
d.c.	dorsal commissure	*s.c.*	scaly cuticle
d.e.v.	dorsal excretory vessel	*s.c.m.*	superficial circular muscles
d.l.m.	deep longitudinal muscles	*s.l.m.*	superficial longitudinal muscles
d.n.	dorsal nerve	*t.*	testis
d.s.n.	dorsal subsidiary nerve	*t.y.d.*	transverse yolk duct
e.v.	excretory vessel	*ut.*	uterus
f.	folds of scolex	*ut.d.*	uterine duct
fl.	posterior flap of segment	*v.d.*	vas deferens
g.a.	genital atrium	*v.e.v.*	ventral excretory vessel
l.c.	longitudinal commissure	*v.ef.*	vas eferens
me.	medulla	*v.c.*	ventral commissure
m.c.	median commissure	*v.n.*	ventral nerve
m.g.	Mehlis's gland	*vag.*	vagina
m.y.d.	median yolk duct	*v.s.n.*	ventral subsidiary nerve
n.	neck	*y.*	yolk gland

REFERENCES

BAER, J. G. (1952). *Ecology of animal parasites.* University of Illinois Press, pp. 1–224.

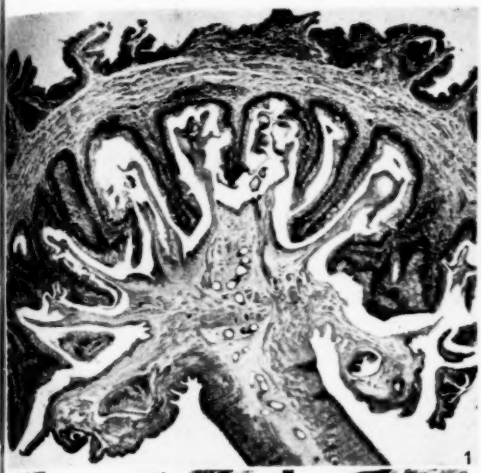
BEAUCHAMP, P. M. (1905). Étude sur les cestodes des sélaciens. *Arch. Parasitol.* **9**, 463–539.

BENEDEN, P. J. VAN (1849). Les helminthes cestoïdes considérés sous le rapport de leurs métamorphoses, de leur composition anatomique et de leur classification. *Bull. Acad. Belg. Cl. Sci.* **16**, 269–82.

BENEDEN, P. J. VAN (1850*a*). Les helminthes cestoïdes considérés sous le rapport de leurs métamorphoses, de leur composition anatomique et de leur classification. *Bull. Acad. Belg. Cl. Sci.*, **17**, 102–8.

BENEDEN, P. J. VAN (1850*b*). Recherches sur le faune littorale de Belgique; les vers cestoïdes. *Mém. Acad. Roy. Belg.* **25**, 1–204.

BENEDEN, P. J. VAN (1858–61). Mémoire sur les vers intestinaux. Suppl. to *C.R. Acad. Sci., Paris*, **2**, 1-376.



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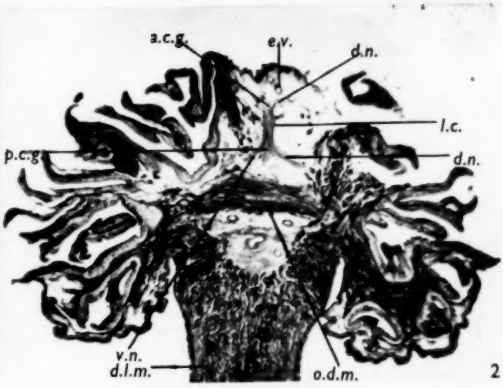

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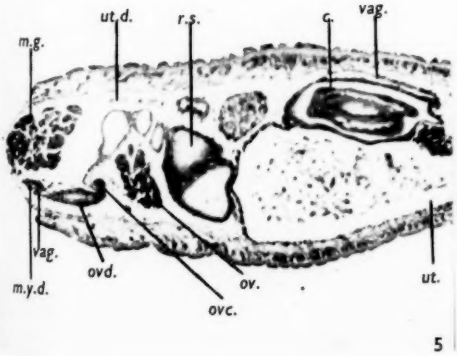

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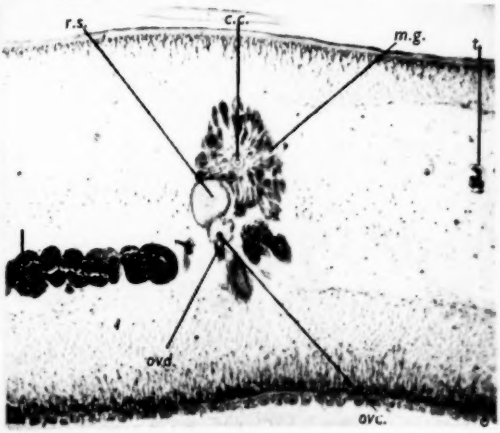

(*Facing p.* 68)

BORCEA, L. (1934). Note préliminaire sur les cestodes des Elasmobranches ou Sélaciens de la Mer Noire. *Ann. sci. Univ. Jassy*, **19**, 345–69.

BRAUN, M. (1894–1900). *H. G. Bronn, Klassen und Ordnungen des Thierreichs*, **4**, Vermes, Abt. 1*b*; Cestodes, 927–1731. Leipzig.

HYMAN, L. H. (1951). *The Invertebrates*. Vol. 2. *Platyhelminthes and Rhynchocoela*, pp. 1–550. McGraw Hill Publication.

JOYEUX, CH. & BAER, J. G. (1936). Cestodes. *Faune Fr.*, **30**, 1–613, Paris.

LINTON, E. (1897). Notes on larval cestode parasites of fishes. *Proc. U.S. Nat. Mus.* **20**, 423–56.

LINTON, E. (1924). Notes on cestode parasites of sharks and rays. *Proc. U.S. Nat. Mus.* **64**, 1–114.

PERRENOUD, W. (1931). Recherches anatomiques et histologiques sur quelques cestodes de Sélaciens. *Rev. suisse Zool.* **38**, 469–555.

PINTNER, T. (1934). Über Entwicklungsvorgänge in der Cestodenkette. *Anz. Akad. Wiss., Wien*, **71**, 256–8.

POCHE, F. (1925). Das System Platodaria. *Arch. Naturg.* **91**, 1–458.

REES, G. (1946). The anatomy of *Phyllobothrium dohrnii* (Oerley) from *Hexanchus griseus* (Gmelin). *Parasitology*, **37**, 163–71.

RISER, N. W. (1955). Studies on cestode parasites of sharks and rays. *J. Tenn. Acad. Sci.* **30**, no. 4, 265–312.

SOUTHWELL, T. (1925). A monograph on the Tetraphyllidea. *Mem. Lpool Sch. Trop. Med.* no. 2. Liverpool University Press.

SOUTHWELL, T. (1929). On the classification of the Cestoda. *Ceylon J. Sci.* **15**, 49–72.

SOUTHWELL, T. (1930). Cestoda. Vol. I. *The Fauna of British India*, 391 pp.

WARDLE, R. A. & MCLEOD, J. A. (1952). *The Zoology of Tapeworms*, pp. 1–780. Minneapolis: University of Minnesota Press.

WOODLAND, W. N. F. (1927). A revised classification of the Tetraphyllidean Cestoda with descriptions of some Phyllobothriidae from Plymouth. *Proc. Zool. Soc. Lond.* 519–42.

YOSHIDA, S. (1917). Some cestodes from Japanese selachians. *Parasitology*, **9**, 560–92.

EXPLANATION OF PLATE II

Key to lettering

a.c.g.	anterior cerebral ganglion	*ov.*	ovary
c.	cirrus	*ovc.*	ovicapt
c.c.	central chamber of Mehlis's gland	*ovd.*	oviduct
d.e.v.	dorsal excretory vessel	*p.c.g.*	posterior cerebral ganglion
d.l.m.	deep longitudinal muscles	*r.s.*	receptaculum seminis
d.n.	dorsal nerve	*t.*	testis
e.v.	excretory vessel	*ut.*	uterus
l.c.	longitudinal commissure	*ut.d.*	uterine duct
m.c.	median commissure	*v.e.v.*	ventral excretory pore
m.g.	Mehlis's gland	*v.n.*	ventral nerve
m.y.d.	median yolk duct	*vag.*	vagina
o.d.m.	oblique deep muscles of scolex		

Fig. 1. *Phyllobothrium sinuosiceps* sp.nov., vertical section through scolex attached to the mucosa of the spiral valve.

Fig. 2. Vertical section through scolex, showing deep oblique muscles, excretory system and 'brain'.

Fig. 3. Transverse section through scolex slightly posterior to the 'brain', showing deep oblique muscles, deep longitudinal muscles and excretory vessels.

Fig. 4. Transverse section through scolex, showing 'brain' and excretory vessels.

Fig. 5. Median sagittal section through posterior region of a free segment, showing relative positions of the male and female genitalia.

Fig. 6. Transverse section through posterior region of free segment, showing female genitalia.

(*MS. received for publication* 2. XII. 1957.—Ed.)

THE LIFE HISTORY AND PATHOGENICITY OF *EIMERIA MELEAGRIMITIS* TYZZER 1929, IN THE TURKEY POULT

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(With 6 Figures in the Text)

INTRODUCTION

In the U.S.A. seven species of *Eimeria* have been described in the turkey, and a previous paper (Clarkson, 1958) has dealt with the life cycle and pathogenicity of one of them, *E. adenoeides*, in Great Britain. This paper deals with a second species, *E. meleagrimitis*.

Tyzzer (1929) gave a brief description of *E. meleagrimitis*, dealing particularly with the morphology of the oocysts and its similarity to *E. mitis* in the chicken. He stated that the site of infection was the small intestine, particularly the lower portion, and that the prepatent period was 6 days. He also gave a brief description of some of the developmental stages. The species was not considered to be very pathogenic as only two deaths occurred in the course of many experiments.

Hawkins (1952) studied the coccidia of the turkey in much greater detail. He described the tissue stages, pathogenicity and pathology of *E. meleagrimitis* infection and stated that this species was the only one which had serious effects on poults and produced 100% mortality in young birds.

There have been brief general references to *E. meleagrimitis* in Great Britain as causing disease in the field, but the species was identified merely by the site of development in the intestine and oocyst measurements (Wilson, 1951; Ford, 1956).

In a paper dealing mainly with clinical coccidiosis in turkeys (Clarkson & Gentles, 1958), the pathology and pathogenicity of *E. meleagrimitis* was briefly described. In the present paper a detailed account is given of the life cycle and host-parasite relationships of a pure strain of this species.

MATERIALS AND METHODS

These were essentially the same as used in the study of *E. adenoeides* (Clarkson, 1958) which should be consulted for details. Coccidia-free poults were used throughout the study and a pure strain of *E. meleagrimitis* was obtained by the selection of a single oocyst and subsequent passage through poults.

The oocyst was obtained from a clinical case of coccidiosis, in which the lesions did not resemble those of *E. adenoeides* and were mainly confined to the duodenum and upper intestine.

For the study of the life cycle, sections and smears were prepared from twelve sites throughout the intestine.

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RESULTS

(*a*) *Life cycle*

Localization of stages in the intestine. The asexual generations occur mainly in the upper small intestine, the greatest concentration being in the upper jejunum, with extension up into the duodenum and down to the region of the rudimentary yolk stalk. Each succeeding stage of development shows a progressively greater spread. Thus, sexual stages may be found in almost any part of the intestine, including the caecum and rectum (Fig. 1). The greatest concentration of gametocytes, however, is in the duodenum and upper jejunum. The sporozoites invade

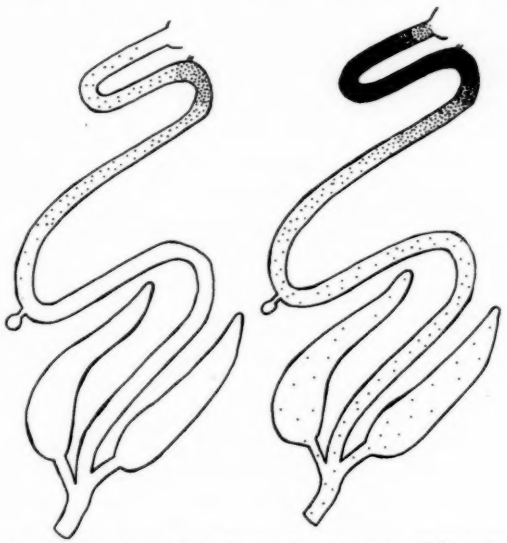

Fig. 1. Localization of first asexual and sexual generation of *E. meleagrimitis* in the intestine of the turkey.

the tips of the villi, but migrate down the villi in the lamina propria to reach the glands. No first-stage schizonts have been seen anywhere except in the glands. This route of entry and migration is the same as that recorded by van Doorninck & Becker (1957) for *E. necatrix* in the chicken. The second-stage schizonts develop in colonies in the deep glands, but also commence to spread up the villi. The third-stage schizonts, the significance of which will be discussed later, occur in all parts of the villi, but never have been found in the glands. The sexual stages are never found in the glands and mainly parasitize the tips of the villi, but also spread down the sides.

Sporozoites (Fig. 2A) were indistinguishable from those of other species of *Eimeria* and exhibit the same types of movement when examined on a warm stage, as has been described for *E. adenoeides*. Smears stained by Giemsa's method showed the nucleus near the centre, several granules in the pointed end, and the posterior rounded part of the sporozoite occupied by a pale-blue 'globule'. As already mentioned, sporozoites entered the cells only at the tips of the villi and

their progress down the villi could be followed in sections up to 24 hr. by the presence of the characteristic 'globule' in their posterior end. Trophozoites (Fig. 2B) developing beneath the cell nucleus were seen after 12 hr. in the gland epithelial cells and by 24 hr. the nucleus of some had commenced to divide (Fig. 2C). By 48 hr. many of the first-stage schizonts were mature, had pushed the nucleus of the enlarged epithelial cell into the gland lumen and contained 80–100 merozoites (Fig. 2D, E). They varied somewhat in size, but were usually about $17 \times 13\ \mu$. Many of the developing first-stage schizonts, which could be distinguished by the 'globule' stained grey with Heidenhain's iron haematoxylin, had undergone only a few divisions and many mature schizonts were found up to 66 hr. and a few even later than this, so that the first asexual cycle was usually completed in 2–2½ days. First-stage merozoites (Fig. 2F) moved by contraction and elongation, their average size in stained smears was about $4{\cdot}5 \times 1{\cdot}5\ \mu$ and the nucleus was situated in the rounded end.

As early as 48 hr. after infection small uninucleate trophozoites (Fig. 2G) were seen developing in small groups or 'colonies', generally in the glands but with some spread up the villi. The colonies were presumably produced by the rupture of a first-stage schizont in the gland, the released merozoites invading a large number of adjacent epithelial cells. The trophozoites developed rapidly and by 66 hr. the majority were mature, measuring $8 \times 7\ \mu$ and containing 8–16 merozoites (Fig. 2H). A few were seen as early as 60 hr. after infection. They were usually situated just beneath the brush border of the epithelial cell, but some developed deep, beneath the cell nucleus, almost on the lamina propria. Thus the second asexual cycle was largely completed by the end of the third day, though a considerable lag occurred as with the first asexual cycle. The second-stage merozoites (Fig. 2I) were larger than the first and measured about $7 \times 1{\cdot}5\ \mu$; the nucleus was near the centre and there were a few small red granules in the pointed end.

In all other *Eimeria* species studied in birds the majority of the second-stage merozoites give rise to the sexual cycle, but in this species a third asexual cycle occurs before any merozoites proceed to develop into gametocytes. Even as early as 72 hr. after infection trophozoites were found along the villi, reaching even to its tip, and third-stage schizonts (Fig. 2J) containing 8–16 merozoites were mature by 96 hr.: a further generation of merozoites was thus produced at this time. These schizonts were of tne same size as the second, but many possessed residual bodies; some of them developed deep to the cell nucleus but the majority were above the nucleus; they were never found in the glands of the mucosa. Third-stage merozoites (Fig. 2K) were of the same size as the second-stage, but the granules in the pointed end were more clearly defined, fewer in number but larger in size, and the nucleus was much nearer the rounded end of the merozoite.

The sexual generation (Fig. 2L) was produced by the invasion of the epithelial cells, particularly at the tips of the villi, by these merozoites. Macrogametes and mature microgametocytes (Fig. 2M) were first found 114 hr. after infection and were of the same general morphology as those of other *Eimeria* species, but considerably smaller than those of *E. adenoeides*. The gametocytes varied in size, but the average was $15 \times 11\ \mu$ compared with $20 \times 18\ \mu$ in *E. adenoeides*. The micro-

gametocyte had a rounded residual mass. The great majority of the gametocytes develop above the nucleus of the parasitized cell. Microgametes (Fig. 2N) were found in large numbers in a bird which died at 6 days and showed two long flagella.

The prepatent period was determined in twenty-five birds by collecting faeces at 2-hourly intervals. The average prepatent period was 116 hr. with a range of

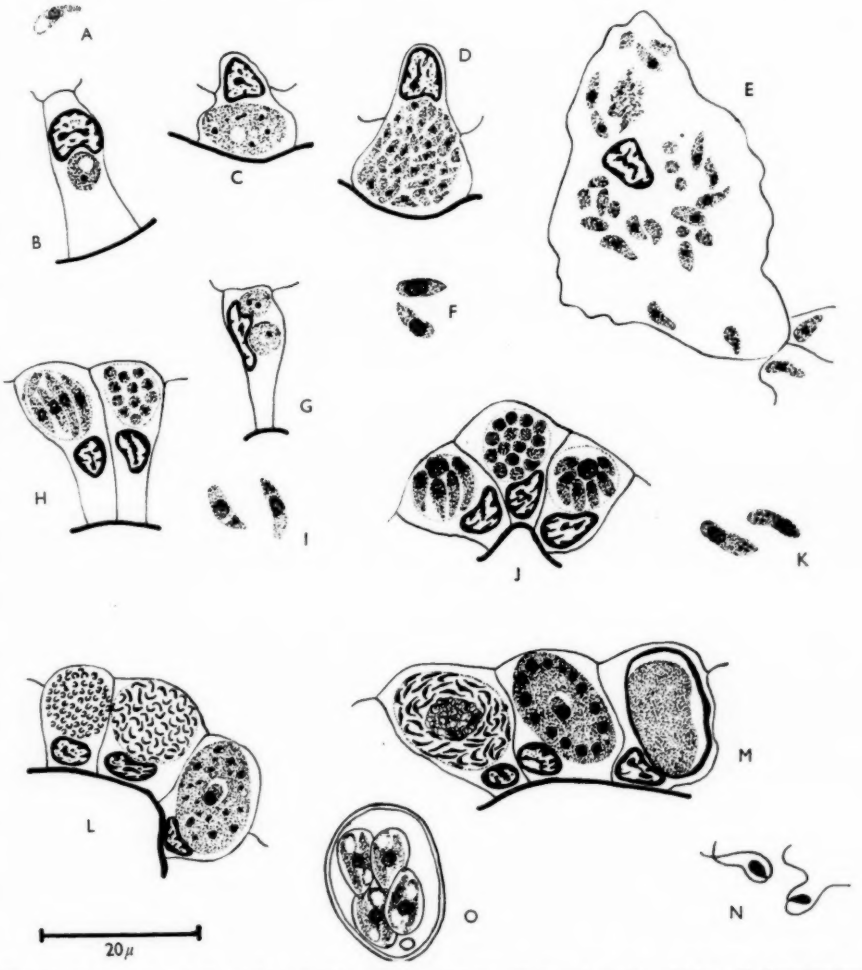

Fig. 2. Life cycle of *E. meleagrimitis*. A, Sporozoite; B, trophozoite: 24 hr.; C, developing schizont: 36 hr.; D, mature first-stage schizont, pushing cell nucleus into gland lumen: 48 hr.; E, ruptured schizont in gland lumen, two merozoites having re-entered cells: 48 hr.; F, first-stage merozoites; G, developing second-stage schizonts: 60 hr.; H, mature second-stage schizonts; I, second-stage merozoites: J, mature third-stage schizonts: 96 hr.; K, third-stage merozoites; L, developing gametocytes: 109 hr.; M, macrogamete, oocyst and mature microgametocyte: 114 hr.; N, microgametes; O, sporulated oocyst. A, F, I, K, and N, Giemsa stained smears; O, fresh preparation; remainder from sections stained with Heidenhain's iron haematoxylin (camera lucida). The thick line represents the base of the epithelial cells.

114–118 hr.; this range is much less than that recorded for *E. adenoeides* under identical experimental conditions.

The oocysts (Fig. 2O) are subspherical. Measurements of 150 sporulated oocysts were $20{\cdot}09 \pm 1{\cdot}95\ \mu$ long and $17{\cdot}33 \pm 1{\cdot}71\ \mu$ wide, the length:width ratio being 1·16:1 (Fig. 3). When placed in 2·5% potassium dichromate, sporulation was complete within 24 hr. at $26 \pm 1°$ C. A refractile granule was present in the fully sporulated oocyst.

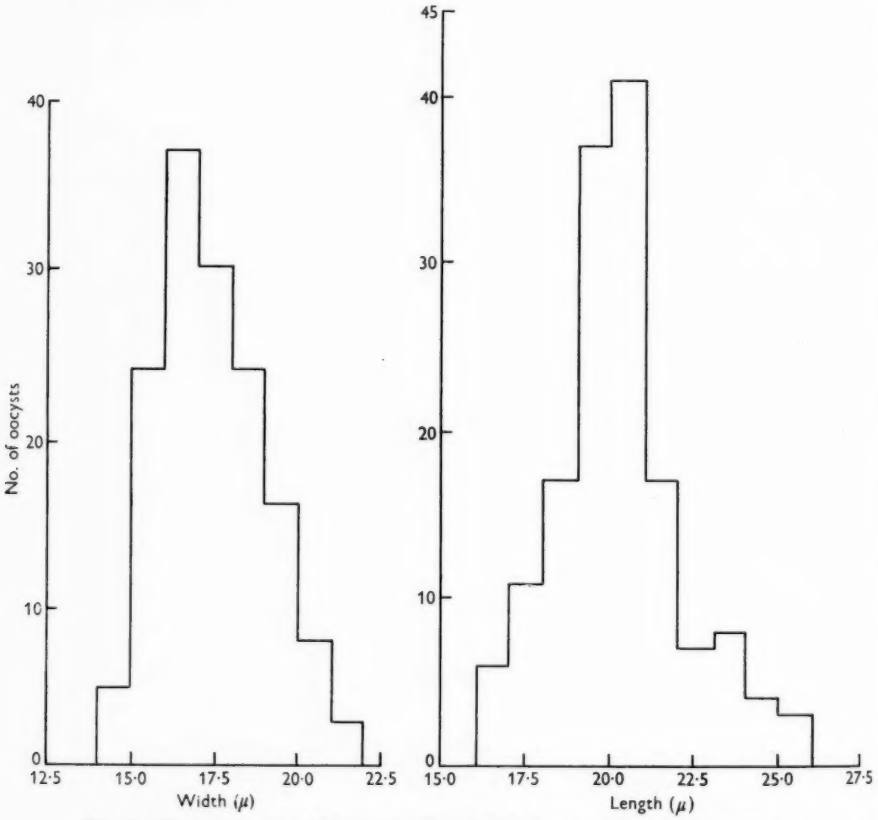

Fig. 3. Histogram of width and length of 150 oocysts of *E. meleagrimitis* in intervals of 1μ.

(*b*) *Pathogenicity*

The symptoms and mortality rate have been briefly presented in a previous paper (Clarkson & Gentles, 1958), and further work has confirmed that *E. meleagrimitis* is pathogenic, causing mortality in young poults up to the age of 6 weeks. As with *E. adenoeides*, an age resistance develops which is not dependent on previous infection (Table 1). Mortality rate is high in birds of about 1–3 weeks of age, but is not usually so high as in *E. adenoeides* infection, although reduction in weight gains occur at all ages and persist longer (Figs. 4–6, Table 2) and infected

Table 1. *Effect of age and size of infective dose on mortality*

Age of poult (weeks)	Dose no. of sporulated (oocysts)	Mortality*
$1\frac{1}{2}$	100,000	5/8
3	100,000	8/22
3	200,000	4/6
4	100,000	0/8
4	300,000	4/10
4	400,000	3/3
5	200,000	0/4
5	1×10^6	2/5
10	2×10^6	0/4

* Numerator: number of birds dying; denominator: number of birds dosed.

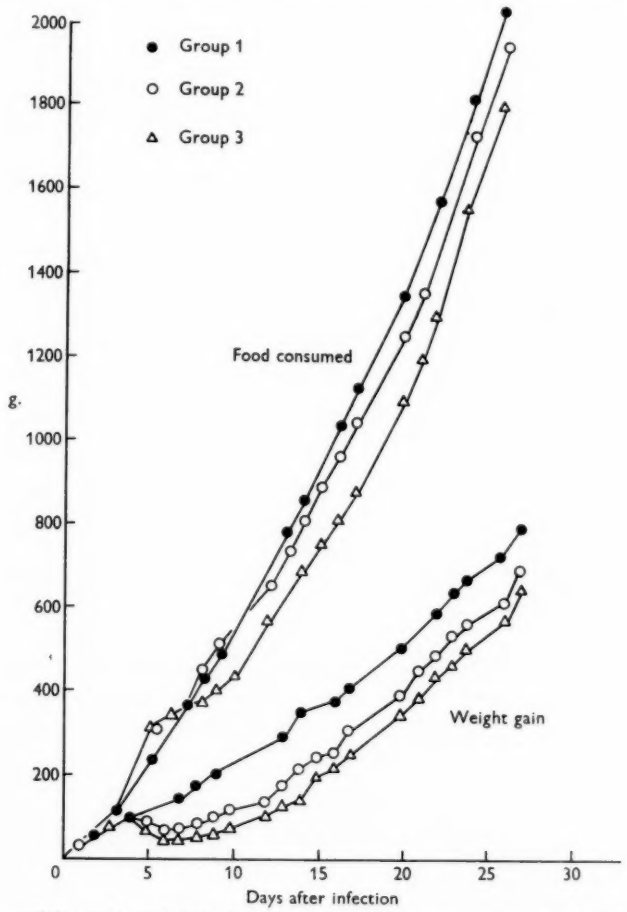

Fig. 4. Mean weight gain and food consumption of three groups, each of eight 3-week-old poults. Group 1: uninfected controls; group 2: infected with 25,000 oocysts; and group 3: with 100,000.

Table 2. *Effect of infection on weight gain (g.) of 3-week-old poults*

Group: 8 birds each	Day of infection		7 days after infection		14 days after infection		22 days after infection		27 days after infection	
	Mean wt.	Range	Mean wt.	Range	Mean wt.	Range	Mean wt.	Range	Mean wt.	Range
Control	203	165–239	352	320–440	551	440–640	792	620–946	995	798–1230
25,000 oocysts	209	166–260	282 $P<0{\cdot}01$*	225–350	428 $P<0{\cdot}01$*	328–534	686 $P<0{\cdot}05$*	530–850	892 $P<0{\cdot}02$*	686–1125
100,000 oocysts	207	168–249	259 $P<0{\cdot}01$*	210–300	378 $P<0{\cdot}01$*	262–474	647 $P<0{\cdot}02$*	480–810	850 $P<0{\cdot}05$*	659–1045

* Significance of difference from control group at same age.

Table 3. *Effect of infection on food conversion*

Food conversion ratio: food/live weight gain

	0–7 days	0–14 days	0–22 days	0–27 days
1. Control	2·52	2·46	2·66	2·86
2. 25,000 oocysts	5·31	3·72	3·07	3·09
3. 100,000 oocysts	6·00	3·74	2·85	2·93

birds never catch up on uninfected controls. Food utilization is reduced in the infected birds (Table 3).

Due to the great difficulty in maintaining large numbers of older birds free from coccidia, the two graphs of the 5- and 7-week-old birds (Figs. 5, 6) do not include an adequate number of controls and are based on only a small number of birds, but as the changes are very marked it seems justifiable to draw some conclusions from them.

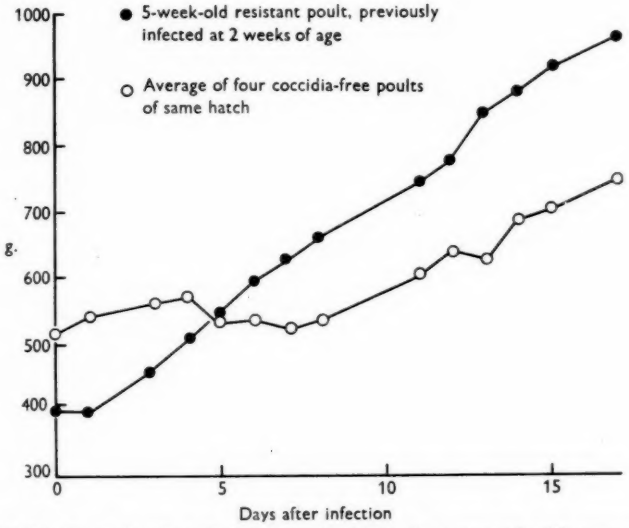

Fig. 5. Weight of previously infected 5-week-old poult compared with average weights of four coccidia-free poults of the same hatch.

Fig. 5 was constructed from an experiment on five birds of the same hatch, four of which were kept coccidia-free, whereas the fifth bird was the survivor of a group of four birds which were infected with 200,000 oocysts at 3 weeks of age, as a result of which it suffered severely from coccidiosis and lost a considerable amount of weight. The five birds were then infected with 200,000 oocysts at five weeks of age, and the average weight of the four previously uninfected birds compared with the weight of the recovered bird. The 'crossing-over' of the graphs showed that the previous infection had rendered this bird resistant, so that it suffered no disease from this second infection. It may thus not be a good thing to maintain birds relatively coccidia-free by husbandry practices since serious weight losses may occur when they meet heavy coccidial infections later on.

Fig. 6 shows the result of infecting 7-week-old birds with four daily doses of 100,000 oocysts, thus simulating field conditions more nearly. The losses in weight extend over a longer period than with a single dose.

(c) *Pathology*

Macroscopic. All the pathological changes were restricted to the intestine, except for loss of condition in birds which suffered a serious attack of the disease

but did not die, in which case the skin was firmly adherent to the underlying breast muscle, which was much darker in appearance than normal, denoting dehydration. No changes were seen in the intestine until the end of the fourth day, when excessive mucus was present containing masses of merozoites and small amounts of blood and other cells. On the fifth and sixth days the duodenum became enlarged and the vessels congested. On opening the duodenum a red-brown necrotic core was seen which, in freshly killed birds, was firmly adherent to the mucosa. This

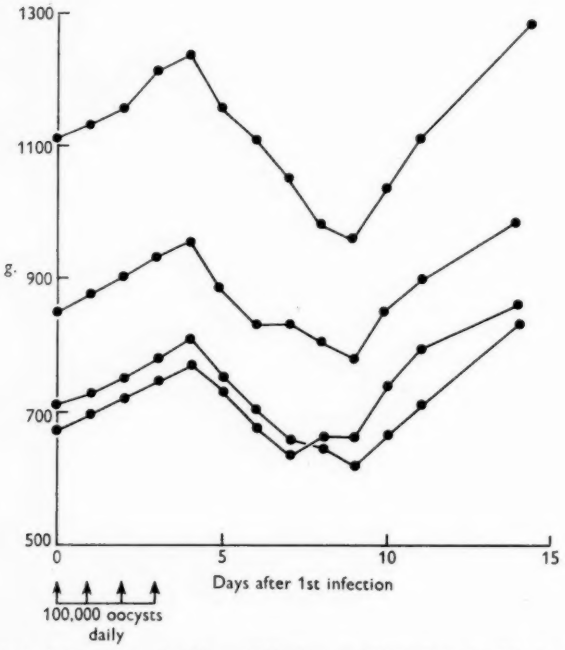

Fig. 6. Weights of four 7-week-old coccidia-free poults infected with four daily doses of 100,000 oocysts.

core extended a small way into the upper small intestine. The remainder of the intestine was congested and most of the small intestine showed petechial haemorrhages in the mucosa which were not visible through the serosa. On the seventh day rapid regeneration of the duodenal epithelium occurred, so that it appeared normal macroscopically but the necrotic material could often be found further down the intestine or in the faeces.

Microscopic. As in the case of *E. adenoeides*, the host reacts to infection with *E. meleagrimitis* by a local eosinophilic infiltration of all parts of the intestine within 2 hr. of infection, and this reaction continues throughout the course of the infection so far as it has been studied—i.e. up to 10 days from first infection. The maximum numbers of eosinophiles were observed in birds which had been infected about 24–48 hr.

Other pathological changes were not marked until late in the life-cycle, when

the bird was seriously affected with clinical disease and they were associated with the development of the sexual stage of the parasite late on the fifth day after infection. The lesions develop with great rapidity, so that sections of the duodenum of birds heavily infected at 96 hr. showed no striking abnormalities except the developing parasites, whereas by 120 hr. profound pathological changes were present. Many of the infected villi gave the appearance of having lost their tips so that the lumen of the duodenum contained masses of developing stages, epithelial cells and considerable numbers of red blood cells. All the blood vessels of this region were congested; many of the epithelial cells around the villi were pale staining and appeared necrotic, and numerous bacteria were seen in the necrotic tissue in the lumen of the intestine. These changes were found in birds which died on the sixth or seventh day from infection, but resolution rapidly occurred in recovered birds, and by the eighth day very little abnormality could be seen, except an increased cellularity of the lamina propria. No extensive fibrosis or thickening of the intestine was seen.

DISCUSSION

In order to identify this species of *Eimeria* as *E. meleagrimitis* it has been necessary to compare the description given by workers in the U.S.A. with the characters described in this paper.

From the morphology of the oocysts there appears no doubt that this species is *E. meleagrimitis* since it is the only one so far described which has a subspherical or ovoid oocyst with a refractile granule when sporulated. The measurements of the oocysts are fairly close to those given by Tyzzer (1929) and Hawkins (1952). The subspherical oocysts of *E. innocua* and *E. subrotunda* do not possess a refractile granule (Moore, 1954).

When comparing Tyzzer's original brief description with Hawkins's more complete study, however, several points of difference occur and my results are not in absolute agreement with either worker. Thus Tyzzer stated that development occurred throughout the small intestine but was greatest in the lowest portion. Hawkins found that the jejunum (i.e. anterior to the rudimentary yolk stalk) was more heavily parasitized than other sites, though development occurred in every part of the intestine, but he does say, however, that in heavy infections much of the epithelium of the jejunum is removed and then the sexual stages develop in the duodenum or ileum. I found that the sexual stages in a heavy infection could develop in any part of the small or large intestine, but that the anterior part of the intestine, especially the duodenum, was most heavily parasitized, even in light infections.

The prepatent period is considered to be of importance in determining specificity, and both Tyzzer and Hawkins found this to be 6 days, although the latter worker had one group of birds with a prepatent period of only just over 5 days. The average prepatent period of my species was 116 hr. but the longest was only 118 hr. This difference does not appear to be of great importance, however, as recent work on other *Eimeria* species has reduced the prepatent period by a day on earlier descriptions (Edgar, 1955; Davies, 1956). It is also obvious that the accuracy with

which the prepatent period can be determined depends on the frequency of faeces collection. Hawkins found the sporulation time of *E. meleagrimitis* to be 48 hr. at 'room temperature', whereas I found almost 100% sporulation in only 24 hr. at 26° C. This difference is obviously of no significance, since temperature profoundly affects sporulation time (Marquardt, 1957; Pérard, 1925). Tyzzer did not state the sporulation time of his strain.

It is when one considers the tissue stages that the most important differences occur. Tyzzer did not describe these in any detail, merely stating that the schizonts measure 6–9·5 by 5·8–7·7 μ (i.e. small) and usually produced about sixteen relatively short and thick merozoites. Hawkins's description agrees with this, but he showed that there were two asexual generations, the schizonts of which were morphologically indistinguishable and were small, containing about twelve merozoites. He also described a slightly larger third-stage schizont occurring with the sexual generation.

The present work shows that there are three distinct asexual generations before the commencement of the sexual generation. In many *Eimeria* species there are only two schizogony cycles before the formation of gametocytes (Tyzzer, 1929; Tyzzer, Theiler & Jones, 1932), though Schaudinn (1900), in the first complete description of the life cycle of an *Eimeria* species—*E. schubergi* in the centipede—considered that there were probably four or five such asexual generations, and Carvalho (1944) described four schizogony cycles of *E. neoleporis* in the rabbit. In other species of *Eimeria*, however, only one schizogony cycle occurs (Hammond, Bowman, Davis & Simms, 1946: *E. bovis*; Lotze, 1953: *E. arloingi*). Finally, the exact number of asexual generations in some species of *Eimeria* could not be determined (Lapage, 1940: *E. caviae*; Davis & Bowman, 1957: *E. zürnii*). One can only conclude that in the genus *Eimeria* there is no fixed number of schizogony cycles before the commencement of sporogony and that the number varies with the particular species concerned.

However, the differences reported in the present work compared with Hawkins's description need to be considered. The second- and third-stage schizonts of my species correspond closely to the first and second of Hawkins's, except that he states that no stages were ever found in the glands, whereas I frequently found groups of second-stage schizonts in the glands. My first-stage schizont is mature by 48 hr., and was found only in the glands. Hawkins first found parasites in a bird killed at 47 hr., although birds infected for shorter periods were examined. One wonders if the first-stage schizonts were missed by Hawkins as he only found uni- or binucleate forms at 47 hr., which would seem very late for the development of the first asexual generation. Many of my sections show these stages belonging to the second generation at 48 hr., and it is very easy to miss the first-stage schizont unless large doses of sporulated oocysts are given. The first-stage schizont can be distinguished by the presence of the refractile 'globule' which has also been described in *E. adenoeides* and other species (Tyzzer, 1929; Lotze, 1953), so there is no doubt that the first generation in my strain of *E. meleagrimitis* is completed by 48 hr. The point arises as to whether there are actually two asexual generations after this one and before the sexual generation, since the two schizonts are morphologically very similar. Slight morphological differences have been noticed, such

as the presence of a residual mass in many of the third-stage schizonts and the position of the nucleus and the size of the granules in the merozoites, but natural biological variation may account for these differences.

In other *Eimeria* species there appears to be some localization of stages along a *villus* as well as along the intestine. Moore & Brown (1951), for instance, used this difference in site of development as one of the reasons for describing *E. adenoides* as a new species, its sexual generation being found in the glands as well as the villi. My experience of *E. meleagrimitis* shows that the second stage occurs commonly in the glands, whereas the third stage is always along the villi and never in the glands. The time relationships of the different stages also support the view that there are three asexual generations before the sexual generation. Thus the peak of first-stage merozoites occurs at 48 hr., the second at 66 hr., and the third at 96 hr. Small second-stage schizonts containing mature merozoites were even seen as early as 60 hr. and it would seem most unlikely that they would not give rise to mature sexual stages until 54 hr. later (114 hr. after dosing).

Confirmatory evidence of the presence of three asexual generations was obtained by counting the number of oocysts produced by feeding a known small number of oocysts. Three birds were each given 800 sporulated oocysts and the oocyst output counted from the fifth to the twelfth day, by which time very few oocysts were being passed. One bird passed some 31 million oocysts, whilst the other two passed 24 million each, an average of 33,000 oocysts for each oocyst fed. When one considers that some oocysts may not have excysted, considerable numbers of merozoites would be lost in the faeces and that not every oocyst would be recovered from the faeces, oocyst output must be even greater than the figures suggest. Two generations of merozoites would be insufficient to produce this number of oocysts even if the maximum number of merozoites in each generation are used to make the calculation (100,16).

There would thus appear to be abundant evidence that in *E. meleagrimitis* three asexual generations occur before the sexual generation.

The situation of the parasite in the cell, particularly in relation to the cell nucleus, is another feature which Tyzzer used in determining specificity. In the case of *E. meleagrimitis*, Tyzzer mentioned that many of the schizonts are found beneath the cell nucleus, though Hawkins found the sexual stages only developed deep beneath the cell nucleus. I found the first-stage schizont was always beneath the cell nucleus, whereas in the succeeding stages the great majority were above it. Reports on the pathogenicity of *E. meleagrimitis* are at variance. Whereas Tyzzer did not consider *E. meleagrimitis* to be very pathogenic, Hawkins (1952) and Moore (1954) reported a high mortality in young poults. I found *E. meleagrimitis* to be pathogenic for young birds but, as in the case of *E. adenoeides*, an age resistance develops which is not dependent on previous infection.

Consideration of all these points of similarity and difference and the use of Tyzzer's criteria for identifying species of *Eimeria* (Tyzzer, 1928) leaves no doubt that the species described in this paper is *E. meleagrimitis*. As in the case of *E. adenoeides* I was unable to obtain a strain from the U.S.A. to compare with my own strain by cross-immunity studies.

SUMMARY

The life cycle and pathogenicity are described of a pure strain of *Eimeria* isolated from turkey poults in Great Britain and identified as *E. meleagrimitis*.

There were three schizogony cycles followed by a sexual generation.

The coccidium produced a high mortality and loss of weight in young poults. An age resistance developed by the time the poults were 8–10 weeks of age.

The pathology of the infection is described.

I would like to thank Professor E. G. White for his guidance throughout this work and for criticism of the manuscript and Miss Moya Gentles for valuable assistance. This work was carried out with the aid of a grant from the Agricultural Research Council.

REFERENCES

CARVALHO, J. C. M. (1944). The coccidia of wild rabbits of Iowa. II. Experimental studies with *Eimeria neoleporis* Carvalho, 1942. *Iowa St. Coll. J. Sci.* **18**, 177–89.

CLARKSON, M. J. (1958). The life history and pathogenicity of *Eimeria adenoeides* Moore & Brown, 1951, in the turkey poult. *Parasitology*, **48**, 70–88.

CLARKSON, M. J. & GENTLES, M. A. (1958). Coccidiosis in turkeys. *Vet. Rec.* **70**, 211–14.

DAVIES, S. F. M. (1956). Intestinal coccidiosis in chickens caused by *Eimeria necatrix*. *Vet. Rec.* **68**, 853–7.

DAVIS, L. R. & BOWMAN, G. W. (1957). The endogenous development of *Eimeria zürnii*, a pathogenic coccidium of cattle. *Amer. J. Vet. Res.* **18**, 569–74.

EDGAR, S. A. (1955). Sporulation of oocysts at specific temperatures and notes on the prepatent periods of several species of avian coccidia. *J. Parasit.* **41**, 214–16.

FORD, E. J. H. (1956). A survey of turkey mortality. *Brit. Vet. J.* **112**, 3–13.

HAMMOND, D. M., BOWMAN, G. W., DAVIS, L. R. & SIMMS, B. T. (1946). The endogenous phase of the life cycle of *Eimeria bovis*. *J. Parasit.* **32**, 409–27.

HAWKINS, P. A. (1952). Coccidiosis in turkeys. *Tech. Bull. Mich. St. Coll.* no. 226.

LAPAGE, G. (1940). The study of coccidiosis in the guinea pig. *Vet. J.* **96**, 144–54, 190–202, 242–54, 280–95.

LOTZE, J. C. (1953). Life history of the coccidian parasite, *Eimeria arloingi*, in domestic sheep. *Amer. J. Vet. Res.* **14**, 86–95.

MARQUARDT, W. C. (1957). The effect of temperature on the sporulation of *Eimeria zürnii* of cattle. *J. Protozool.* **4** (suppl.), 10.

MOORE, E. N. (1954). Species of coccidia affecting turkeys. *Proc. 91st Annual Meeting Amer. Vet. Med. Ass.*, pp. 300–3.

MOORE, E. N. & BROWN, J. A. (1951). A new coccidium pathogenic for turkeys, *Eimeria adenoeides* n. sp. (Protozoa: Eimeriidae). *Cornell Vet.* **41**, 125–36.

PÉRARD, C. (1925). Recherches sur les coccidioses du lapin. II. Contribution à l'étude de la biologie des oocystes des coccidies. *Ann. Inst. Pasteur, Paris*, **39**, 505–42.

SCHAUDINN, T. (1900). Untersuchungen über den Generationswechsel bei Coccidien. *Zool. J. Anat.* **13**, 197–292.

TYZZER, E. E. (1928). Methods for isolating and differentiating species of *Eimeria* occurring in gallinaceous birds. *J. Parasit.* **15**, 148–9.

TYZZER, E. E. (1929). Coccidiosis in gallinaceous birds. *Amer. J. Hyg.* **10**, 269–384.

TYZZER, E. E., THEILER, H. & JONES, E. E. (1932). Coccidiosis in gallinaceous birds. II. A comparative study of species of *Eimeria* in the chicken. *Amer. J. Hyg.* **15**, 319–93.

VAN DOORNINCK, W. M. & BECKER, E. R. (1957). Transport of sporozoites of *Eimeria necatrix* in macrophages. *J. Parasit.* **43**, 40–4.

WILSON, J. E. (1951). Sulphaquinoxaline and sulphamezathine in the treatment of experimentally induced caecal coccidiosis of chickens (*E. tenella*) and in a natural outbreak of coccidiosis in turkeys (*E. meleagridis* and *E. meleagrimitis*). *Vet. Rec.* **63**, 373–7.

TAXONOMY, LIFE CYCLE, DEVELOPMENT, AND HABITS OF TWO NEW SPECIES OF ALLANTONEMATIDAE (NEMATODA) PARASITIC IN DROSOPHILID FLIES*

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(With 33 Figures in the Text)

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I. INTRODUCTION

There are few records in the literature of internal parasitism of drosophilids by nematodes. Basden & Goodey (1955) cited three and added a fourth by recording the parasitism of fruit flies at Edinburgh, Scotland, by an undescribed species of Allantonematidae Chitw. & Chitw., 1937. Investigations made at Rothamsted Experimental Station, Harpenden, Herts, England, revealed the occurrence of this species, and of another new species, as well as of an unidentified species in six of the twelve species of fruit flies captured.

In preliminary studies in 1954, fruit flies were collected daily in the Manor Woods at Rothamsted with baits of drosophila medium (Demerec, 1948). Collections were made weekly from 1 January 1955 to 1 October 1956, from traps of a design used by Basden (1954). Most of the flies were identified by the author, but doubtful cases were sent to Mr E. B. Basden, Edinburgh, for confirmation. Flies were

* Contribution No. 3755, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada; in part from a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of London, 1956.

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dissected in Ringer's solution and the nematodes fixed in Ditlevson's mixture or in the neutralized formalin mixture of Courtney, Polley & Miller (1955), stained in cotton blue lactophenol (Franklin & Goodey, 1949), and mounted by Baker's (1953) method.

II. *HOWARDULA AORONYMPHIUM* SP.NOV.

*Description**

Adult female. Holotype: *L* 1·88 mm.; *W* 0·164 mm.; *a* 11·5; *EL* 46–59 μ; *EW* 34–36 μ. Paratypes: eggs in uterus; *L* 1·28–1·52 mm., av. 1·39 mm.; *W* 0·152–0·163 mm., av. 0·158 mm.; *a* 8·4–9·3; *n* 3; eggs and larvae in uterus; *L* 1·31–4·41 mm., av. 2·05 mm.; *W* 0·127–0·279 mm., av. 0·201 mm.; *a* 6·4–15; *n* 14.

Head broadly rounded. Body of equal width throughout its length, but tapering to rounded tail tip (Fig. 1); usually C-shaped, often with head and tail overlapping. Cuticle thicker on head and tail (4–6 μ) than on body (2–3 μ), and smooth though slightly folded in tail region. Lateral fields, lip structures, stoma, and stylet not visible. Structures in head degenerate and crowded together. Paired oval bodies (lateral structures of Thorne, 1935), with bright refractory centres, 21–25 by 17–19 μ in diameter and situated 20 μ below cuticle, with which they have no visible connexion, and 0·11 mm. from the head. Gonad single, with two reflexes separating the uterus, oviduct, and ovary; ovary with small reflex in head; main portion of ovary and oviduct two-fifths as long as body; triangular-shaped receptaculum seminis in head; uterus extending from head to tail and filling posterior three-fifths of the body. Eggs and larval nematodes present. Vulva and anus not visible. Tail with several large cells with clearly defined nuclei. Worm often ensheathed with host tissue.

Adult male. Allotype: *L* 0·461 mm.; *W* 0·015 mm.; *a* 31; *c* 7·5; *T* 61 %. Paratypes: *L* 0·361–0·475 mm., av. 0·444 mm.; *W* 0·012–0·016 mm., av. 0·015 mm.; *T* 53–66 %; *a* 28–34; *c* 7·0–8·7; *n* 23.

Body tapering anteriorly from region of excretory pore and posteriorly from anal region to tail tip (Fig. 2A). Preserved material translucent under low magnification and curved slightly ventrally. Cuticle finely striated throughout, especially on head, where the striae are less than 1 μ wide. Striae broken by well-defined lateral fields extending from just behind head to tail tip. Lateral field 4 μ wide at middle of body and with several incissures. Deirids in plane of excretory pore. Head rounded and not set off. Lips, papillae, amphids, stoma, and stylet not visible. Vague outline of oesophagus widening and curving as it extends back until displaced by testis. Excretory pore 98 μ from tip of head, the duct extending for a short distance at right angles to cuticle. Faintly defined hemizonid immediately anterior to excretory pore. Intestine with vacuolate structure near excretory pore. Single testis filling most of body and hiding intestine; filled with sperms and tapering to vas deferens. Two spicules each gently curving from broad base to blunt, squarish end. Left spicule 19 μ and right 21 μ long. Bursa and gubernaculum absent. Two swollen cells and traces of tubules present in tail. Tail usually curving

* See p. 102 for explanations of systematic abbreviations.

in preserved specimens; narrowing gradually, with characteristic kink halfway along its length and with tip bulbously expanded (Fig. 2B).

Fourth-stage free-living female. Type: *L* 0·477 mm.; *W* 0·014 mm.; *A* 0·064 mm.; *a* 34; *c* 7·4; *V* 83 %. Paratypes: *L* 0·423–0·477 mm., av. 0·449 mm.; *W* 0·013–0·015 mm., av. 0·014 mm.; *V* 79–83 %; *a* 31–35; *L* of stylet 14–15 μ; *L* of excretory pore from anterior tip of head 0·084–0·104 mm.; *L* of gonad in front of vulva 0·100–0·139 mm.; *L* of gonad behind vulva 3–7 μ; *n* 10.

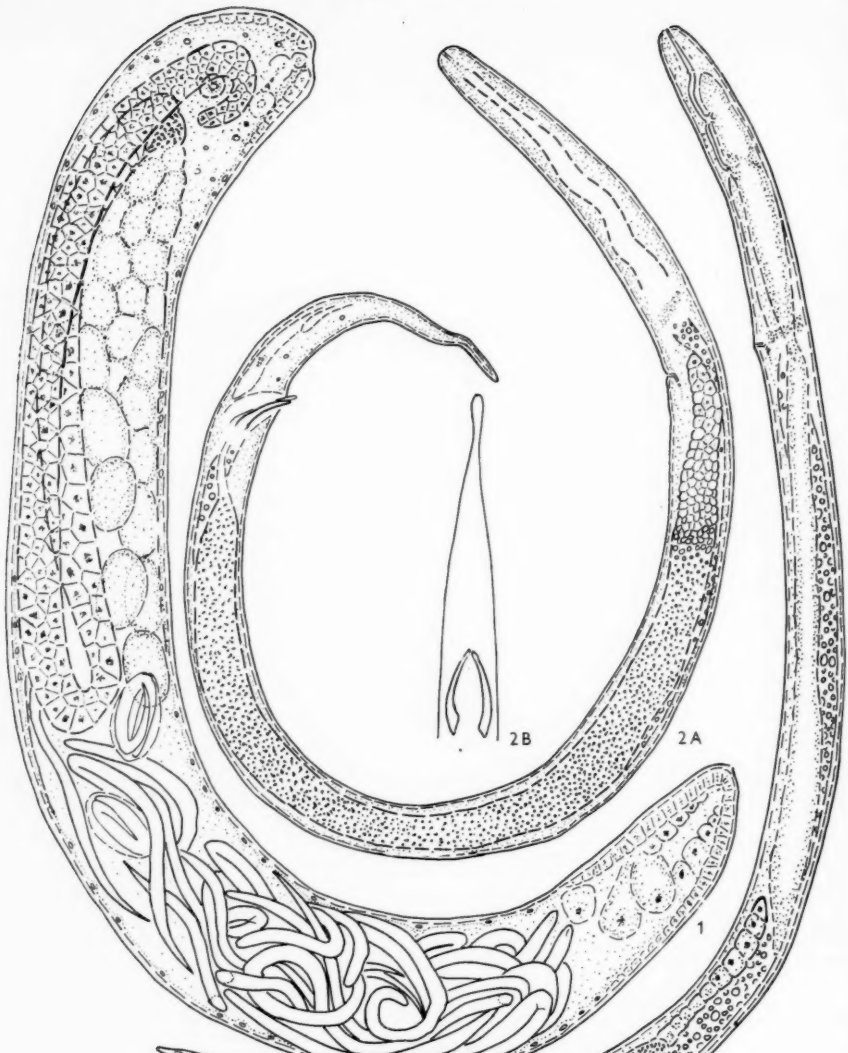
Body tapering gradually from nerve ring to head, and in posterior fourth (Fig. 3). Preserved specimens curved slightly ventrally. Cuticle bearing minute transverse striae, less than a micron wide on head but wider on body. Lateral fields 3–4 μ wide at middle of body. Head not set off; lip region slightly swollen. Stoma visible and mouth terminal. Stylet 12 μ long with conical tip and shaft 1 μ in diameter. Basal knobs not present. Oesophageal lumen (2 μ wide) curves ventrally from base of stylet. Dorsal gland with duct joining slightly swollen oesophageal lumen at 25 μ from tip of head. Oesophageal lumen (1 μ wide) terminating 15 μ farther back in an enlarged junction with ducts of subventral glands. Behind this junction are traces of the vacuolate structure of the intestine. Dorsal gland extending forward to within 14 μ of front of head and appearing to fuse posteriorly with subventral glands. Subventral glands fused, tapering near nerve ring to a quarter of body width, widening again, and terminating ventrally immediately anterior to the ovary. Nerve ring 91 μ from tip of head. Excretory pore 9 μ behind nerve ring, with a short duct visible at right angles to cuticle. Intestine occupying half of body width a short distance behind excretory pore. Ovary ventral, 0·17 mm. from head; 38 μ long and 6 μ wide, consisting of five to seven well-defined cells. Gonad narrowing behind ovary to form oviduct initial of indistinct cells. Uterus thin-walled, filled with sperms, 92 μ long, and with a maximum width of 7 μ. Vulva and vagina difficult to discern, though former often indicated by slight swelling of cuticle. Uterus extending to 12 μ behind vulva. Intestine, with vacuolated structure, displaced by gonad to end of uterus, then crossing to the mid-line. Faint trace of anus 52 μ from tail tip. Cells of unknown structure present in tail. Tail displaying slight kink and tapering to minute, slightly bulbous tip.

Larval stages described below.

Host and locality. The type material was found in the abdominal haemocoele of a specimen of *Drosophila phalerata* Meig. (Diptera: Drosophilidae) trapped in the Manor Woods, Rothamsted Experimental Station, Harpenden, Herts, England. The species was also found in *D. kuntzei* Duda. The holotype and the allotype are in the British Museum (Natural History). Series of paratypes are in the Nematology Collection, Rothamsted Experimental Station, and the Canadian National Collection of Nematodes at Ottawa and Belleville.

Diagnosis. A species of *Howardula* displaying ovoviviparity. Gravid female without visible anus or vulva. C-shaped plump body, 1·5–3·0 mm. long. Male with bursa and gubernaculum lacking or much reduced. Similar to *H. oscinellae*, but male and immature females with longer tail that tapers to a fine point.

Wachek (1955) recognized five species in this genus. Three of these show ovoviviparity: *H. benigna* Cobb, 1921; *H. oscinellae* (Goodey, 1930) Wachek, 1955; and



Figs. 1–4. *Howardula aoronymphium* sp.nov. 1. Adult gravid female from haemocoele of *D. phalerata*. 2A. Free-living adult male. 2B. Ventral view of adult male tail showing spicules. 3. Free-living inseminated larval female 4A. Egg from motherworm. 4B. Embryonated egg.

H. phyllotretae Oldham, 1933. *H. aoronymphium* is most closely related to *H. oscinellae*, which also has a dipteron host. The gubernaculum was not found in *H. aoronymphium* despite numerous detailed examinations.

The genus *Prothallonema* Christie, 1938, was erected for a species taken from decaying fruit, but Christie's description indicates that his species is unrelated to *H. aoronymphium*, despite the probable similarity in hosts.

Life cycle

The life cycle of *H. aoronymphium* is similar to that of most known Allantonematidae. Goodey (1930) described this type of life cycle in detail, but there are differences between the life cycle of his species and that of *H. aoronymphium*.

Though the actual entrance into the host was not observed, it was found that the nematode has a stylet and the dorsal gland was deflated immediately after the entrance. These facts support the hypothesis that the fertilized females enter the host larvae by penetrating the host cuticle. Entry might be achieved through the mouth or the anus, but in numerous dissections of host larvae no nematodes were found in the oesophagus or intestine; those found were all in the host haemocoele.

The inseminated female increases in size, forms eggs, and discharges approximately 500 larvae into the host haemocoele. The larvae move about in the host abdomen, and occasionally in the head and the thorax. Eventually they leave the host through the genital or the intestinal tract, or both. Exit through the genital tract was observed only in female hosts. Goodey (1931) described only the intestinal exit in *H. oscinellae*, whereas Cobb (1921) and Oldham (1933) noted that the exit was made through the genital system in both *H. benigna* and *H. phyllotretae*.

The larvae drop at or near the oviposition sites of the hosts. Examination of material from oviposition sites failed to reveal specimens of free-living stages of this worm in the field. In the laboratory, however, active free-living larvae were found in culture bottles in which wild-caught insects were kept. Before mating and copulation the males undergo the final moult and the females the penultimate moult. Copulation was not observed in tap-water cultures, but apparently took place in culture bottles. The males die after copulation, and the females enter the final host to commence another cycle.

Growth and development

Three moults were observed in the development of the female and two in that of the male. The earliest moult observed in the female, probably the second, occurred in the host body cavity, the third in the free-living state, and the final one in the haemocoele of the definitive host. The time of occurrence of the last moult is unusual; in other known species of this family it takes place in the free-living state. Its occurrence in *H. aoronymphium* indicates that the females are fertilized as fourth-stage larvae. The worm might moult before penetration and cast its skin afterwards, but the penetration process would probably remove the skin. Wachek (1955) also recorded a moult after fertilization in females of several species.

The free-living males are often found enclosed in the larval skins of the third and fourth stages. The third moult probably occurs as the nematode leaves the

host, for larvae are often found in the genital and intestinal tracts of the host with loose cuticle on their head and tail regions. The first and second cast cuticles were not found despite careful examination of the egg and early larval stages. Attempts to rupture the eggs and to extrude the embryo with any loose skins were unsuccessful, because the eggs were crushed by the slightest pressure. A similar apparent loss of moults occurs in other phasmid nematodes; namely, the Ascaroidea, Filarioidea and Syngamidae. In these groups one or two moults may occur while the embryos are in the egg membrane, so that the larvae have fewer than four moults. Raski (1950) found a cast cuticle in the egg of *Heterodera schachtii* Schmidt, 1871, in the Tylenchoidea, and demonstrated four moults where previously only three were known. These facts support a hypothesis that *H. aoronymphium* has the usual four moults with one or two in the egg and two or three during the larval development.

The body lengths of larvae at various stages of development are given in Table 1.

Table 1. *Means and ranges of body lengths (mm.) for stages of growth of* Howardula aoronymphium

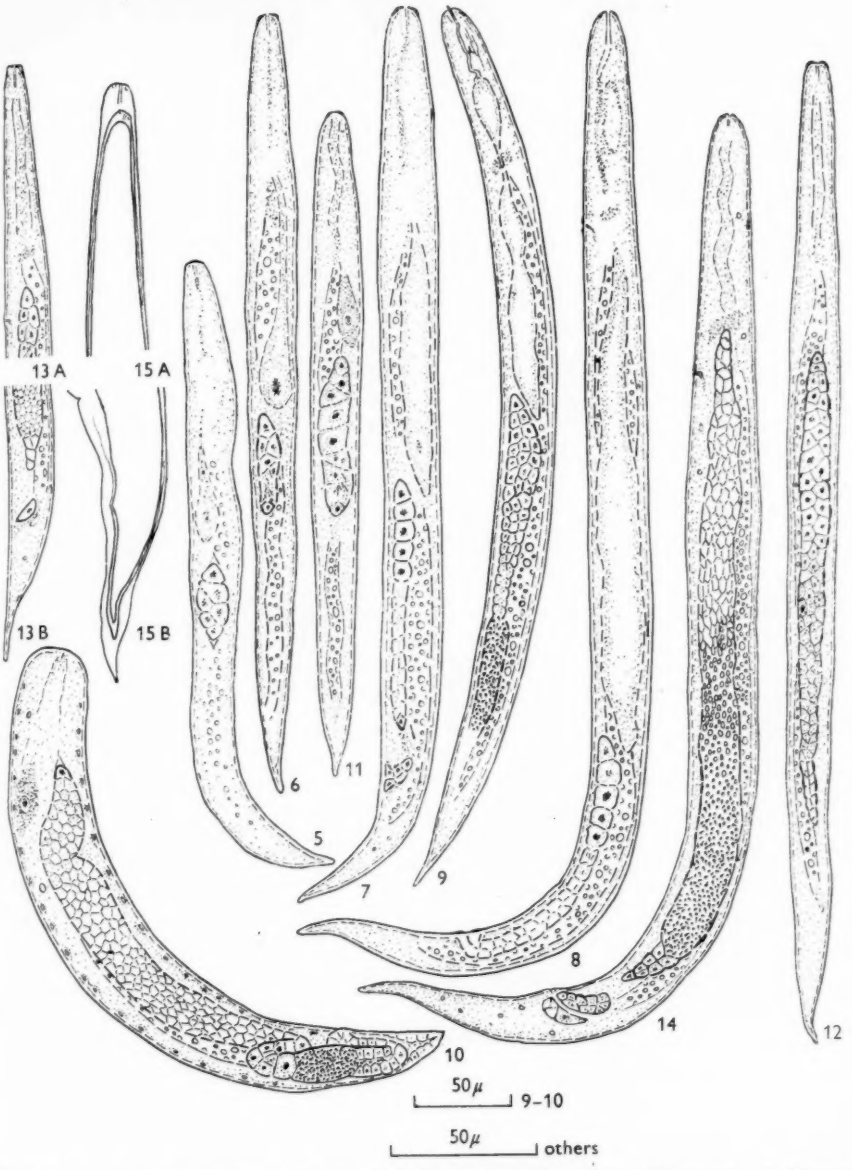
(Each measurement based on ten specimens.)

Stage	Female	Male
From uterus of motherworm	0·218 (0·163–0·252)	0·228 (0·202–0·257)
Second moult	0·233 (0·201–0·285)	—
From host gonads	0·366 (0·341–0·384)	0·350 (0·326–0·369)
Third moult	0·436 (0·417–0·468)	—
Fourth moult	0·407 (0·378–0·447)	—
Free-living adults in 3rd and 4th skins	—	0·377 (0·347–0·411)

Most growth occurs when the worms are parasitic. The decrease in length of the female at the fourth moult is compensated by an increase in width. The lengths of the males found in host gonads are probably reasonable approximations of their lengths at the third moult. There is little difference in the size of the eelworms from the two hosts *Drosophila kuntzei* and *D. phalerata*.

Many of the early stages of egg cleavage can be seen in the uterus of the female. Before hatching, the embryo has at least two flexures that divide the body into three equal lengths (Fig. 4*B*). Embryos are active in the egg and often have more than two flexures. The cells of the genital primordium are sometimes visible in the advanced embryo and there is often an indication of the oesophagus.

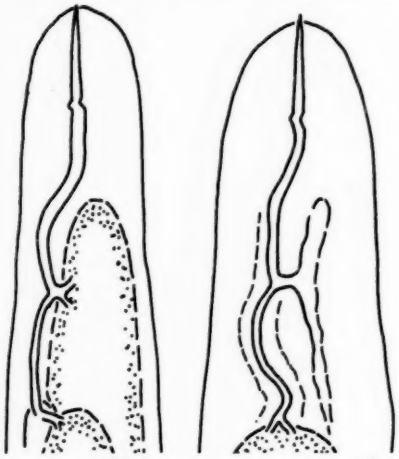
Both the genital and intestinal systems (Figs. 5–17) have a development similar to that described by Goodey (1930) for *H. oscinellae*. The male genital system is more advanced in all larval stages than that of the female, enabling the sex of the larva to be determined in the uterus of the motherworm. In contrast to the fully developed gonad of the male at fertilization, the female gonad consists only of a vulva, vagina, and ovarial primordium of five to ten cells (Fig. 3). Wachek (1955) described this immaturity of the female, found by him in many tylenchid parasites of insects, as koriogamy, and thus restricted Wülker's original definition (1923). After fertilization and host entrance the ovary enlarges to fill the anterior half of



Figs. 5–15. *H. aoronymphium*. 5. Female larva from uterus of motherworm. 6. Female larva from haemocoele of host, *D. phalerata*. 7. Female larva from haemocoele of host, *D. phalerata*. 8. Female larva from uterus of *D. phalerata*. 9. Female larva from host larva. 10. Growing adult from host pupa. 11. Male larva from uterus of motherworm. 12. Male larva from host haemocoele. 13A, B. Head and tail of older male larva from host haemocoele. Note spicular primordium. 14. Larva from uterus of host, 15A, B. Head and tail of moulting adult male.

the worm and the uterus, filled with larvae, occupies the posterior half of the body. The manner in which the larvae leave the female is unknown, as the vulva was not found in the mature gravid female; in several specimens, however, the larvae appeared to have ruptured the cuticle of the motherworm by their great numbers.

The oesophageal glands and associated structures in the female develop gradually, but little of their development can be seen in the male. In a larva from the uterus of the motherworm the gland initial is a single triangular cell situated near the gonad primordium (Fig. 5). When the larvae leave the host, this glandular structure is readily recognized and extends half the body length. A glandular structure also



Figs. 16–17. *H. aoronymphium.* 16. Head of free-living inseminated female larva. 17. Head of this larva showing deflation of gland.

develops in the head and the first portion of the oesophageal lumen is clearly visible. The oesophageal glands and the stylet attain their ultimate development in fertilized females (Figs. 3, 16). The oesophageal lumen is displaced to the ventral side of the body by the enlarged dorsal gland. It is difficult to separate the dorsal gland from the subventral and to distinguish each of the subventral glands (Fig. 16). Two faintly defined nuclei are sometimes visible in the posterior end of the fused subventral glands and provide evidence of their dual nature. The dorsal gland in a maturing female removed from a recently infected larva has almost disappeared (Fig. 17). Its deflation is probably associated with the production of a material for penetration into the final host. The two ducts and dual nature of the subventral glands are more discernible than in earlier stages.

The occurrence of the two oval bodies in the head of the adult females is noteworthy as they closely resemble the numerous lateral structures extending throughout the body of *Aphelenchulus reversus* Thorne, 1935; and this is apparently the first confirmation of Thorne's observation (1935).

III. *PARASITYLENCHUS DIPLOGENUS* SP.NOV.

This species closely resembles *P. dispar typographi* (Fuchs, 1914) Micoletzky, 1922, and *Tylenchus dispar curvidentis* Fuchs, 1914. The generic relationships of these species required careful examination before the genus could be determined. Fuchs described *T. dispar cryphali* and *T. dispar curvidentis* and gave a few descriptive details of *T. dispar typographi* and *T. contortus typographi* in 1914. The publication of full descriptions of these last two species in 1915 has led to confusion as to the exact year of publication, but the 1914 date has priority (see also Stiles & Hassall, 1920). Micoletzky erected the genus *Parasitylenchus* in 1922 and assigned these four forms to it, but did not designate a type. In 1925 he moved these forms to *Aphelenchulus* Cobb, 1920, while in the next year Baylis & Daubney placed them in *Anguillulina* Gerv. & v. Ben., 1859. In 1934 Filipjev retained *Parasitylenchus*, and designated *P. dispar* (Fuchs, 1914) Micoletzky, 1922, the type species of the genus *Parasitylenchus*. Any doubt as to which form Filipjev meant by his designation of *P. dispar* is dispelled by his synonymy of *P. dispar* and *T. dispar typographi* Fuchs, 1915 (Stiles & Hassall (1920) also considered these names synonymous), and his transfer of the other forms to another genus. *P. dispar curvidentis* (Fuchs, 1914), Micoletzky, 1922 and *P. dispar cryphali* (Fuchs, 1914), Micoletzky, 1922, were transferred to *Aphelenchulus* Cobb, 1920. *P. contortus typographi* (Fuchs, 1914) Micoletzky, 1922, was found a synonym of *A. diplogaster* (Linstow, 1890), Filipjev, 1934. In this reorganization he also reduced the trinomial names of Fuchs to binomials, and the first three forms became the species *P. dispar* (Fuchs, 1914) Micoletzky, 1922, *A. curvidentis* (Fuchs, 1914) Filipjev, 1934, and *A. cryphali* (Fuchs, 1914), Filipjev, 1934.

In 1955 Wachek set up three subgenera in the genus *Parasitylenchus*. He accepted *P. dispar* (Fuchs, 1914), Micoletzky, 1922 as the type species for the genus as well as for the typical subgenus. Rühm (1956) recognized the common characteristics of morphology and the two-generation life cycle of *P. dispar* and *P. curvidentis*, and assigned them to a new genus, *Polymorphotylenchus*, though separating them at subgeneric level. He designated *P. typographi* (Fuchs, 1914), Micoletzky, 1922, as type species of this genus and its typical subgenus, and *curvidentis* as type of the other subgenus, *Thylakolenchus*. He also designated *P. cryphali* (Fuchs, 1914), Micoletzky, 1922, as the type species of *Parasitylenchus*.

Rühm's assignment of these two species to one genus is sound. His subgeneric distinction of them is chiefly based on the sac-like body shape displayed by the 'small' (parasitic generation) females in *P. curvidentis* compared with the wormlike form in the same generation of *P. dispar*. A series of females from both generations of *diplogenus* showed the gradual transition from worm-like to sac-like shapes. A sac-like shape is apparently the result of ageing and is therefore of little taxonomic value.

I recognize *P. dispar* as the type species of *Parasitylenchus*, and accordingly consider that *P. curvidentis* and *P. diplogenus* belong to the genus. Rühm's diagnosis of *Polymorphotylenchus* represents an emendation of the original

diagnosis given by Micoletzky for the genus *Parasitylenchus*. The revised classification of the genus is as follows:

Genus **Parasitylenchus** Micoletzky, 1922

Parasitylenchus Micoletzky, 1922; p. 545.
Polymorphotylenchus Rühm, 1956, p. 147.

TYPE. *Parasitylenchus dispar* (Fuchs, 1914), Micoletzky, 1922.

Parasitylenchus dispar (Fuchs, 1914), Micoletzky, 1922

Tylenchus dispar typographi Fuchs, 1914; p. 195.
Parasitylenchus dispar var. *typographi* (Fuchs, 1915), Micoletzky, 1922; p. 545.
Aphelenchulus dispar var. *typographi* (Fuchs, 1915), Micoletzky, 1925; p. 125.
Anguillulina dispar var. *typographi* (Fuchs, 1915), Baylis & Daubney, 1926; p. 65.
Parasitylenchus dispar (Fuchs, 1915), Micoletzky, 1922, *in* Filipjev, 1934; p. 394.
Polymorphotylenchus (*Polymorphotylenchus*) *typographi* (Fuchs, 1915), Rühm, 1956; p. 149.

Parasitylenchus curvidentis (Fuchs, 1914), Micoletzky, 1922

Tylenchus dispar curvidentis Fuchs, 1914, p. 195.
Parasitylenchus dispar var. *curvidentis* (Fuchs, 1914), Micoletzky, 1922; p. 545.
Aphelenchulus dispar var. *curvidentis* (Fuchs, 1914), Micoletzky, 1925; p. 125.
Anguillulina dispar var. *curvidentis* (Fuchs, 1914), Baylis & Daubney, 1926; p. 65.
Aphelenchulus curvidentis (Fuchs, 1914), Micoletzky, 1922, *in* Filipjev, 1934; p. 387.
Polymorphotylenchus (*Thylakolenchus*) *curvidentis* (Fuchs, 1914), Rühm, 1956; p. 154.

Parasitylenchus diplogenus sp.nov.

The twenty-nine species that have been assigned to this genus by Wachek (1955) and Rühm (1956) are not within the concept of the genus as based on the type species *P. dispar* (Fuchs, 1914), Micoletzky, 1922.

Description

Adult female, completely parasitic generation. Holotype: *L* 0·504 mm.; *W* 0·078 mm.; *V* 86%; *A* 0·021 mm.; *a* 6·4; *c* 24; *EL* 39μ; *EW* 21μ. Paratypes: *L* 0·423–0·547 mm., av. 0·483 mm.; *W* 0·076–0·097 mm., av. 0·083 mm.; *A* 0·015–0·042 mm., av. 0·025 mm.; *V* 84–92%; *a* 4·8–6·7; *c* 13–18; stylet length 5–8μ; *n* 21.

Cuticle 1μ thick on head, thinner at middle of body, and 2μ thick at tail. Stoma and stylet not readily visible, especially in older specimens. Stylet 7μ long and 1μ wide at its base. Oesophageal, glandular, and intestinal structures displaced by immense growth of gonad to within 11μ of head tip. Single gonad occupying most of body, and divided by double reflexes into uterus, oviduct, and ovary (Fig. 18). Ovary extending back four-fifths of body length, with small reflex in head. Oviduct extending forward a third of body length. Receptaculum seminis usually dorsal at middle of body. Two to four eggs in uterus. Vagina thick-walled, at right angles to cuticle. Vulva 71μ from tail tip, surrounded by swollen cuticle.

Some intestinal structures visible behind vulva, but difficult to find in most material. Tail containing cells and tissue of unknown structure. Worms loose in host haemocoele.

Adult male, completely parasitic generation. Allotype: *L* 0·284 mm.; *W* 0·018 mm.; *A* 0·027 mm.; *T* 90 %; *a* 15; *c* 10. Paratypes: *L* 0·188–0·290 mm., av. 0·256 mm.; *W* 0·017–0·025 mm., av. 0·021 mm.; *A* 0·018–0·027 mm., av. 0·023 mm.; *T* 88–92 %; *a* 11–17; *c* 8–13; *n* 20.

Vermiform body tapering slightly to rounded lip region, and abruptly to tail tip (Fig. 19). Preserved material often curving, particularly in spicular region, with ventral side outermost. Cuticle smooth, without striae or transverse markings. Lip region slightly swollen. Short stoma present, but stylet absent. Oesophageal and glandular structures degenerate and displaced by testis. Testis (7μ wide) reaching to within 7μ of head tip, and occupying almost the complete body width from 65μ behind head backwards for four-fifths of body length. Vas deferens short. Two separate spicules, right 11μ and left 10μ long, slightly curved, in preserved material projecting unevenly from cloaca, and each with an enlarged base (2μ wide) followed by a slight constriction of the shank and a gradual taper in a smooth curve to a truncate end. Bursa and gubernaculum absent. Tail tip pointed.

Adult female, partially free-living generation. Type: *L* 0·304 mm.; *W* 0·063 mm.; *V* 89 %; *a* 4·8; *EL* 38μ; *EW* 26μ. Paratypes: *L* 0·224–0·396 mm., av. 0·238 mm.; *W* 0·033–0·063 mm., av. 0·052 mm.; *V* 83–91 %; *a* 3·7–12; *n* 13.

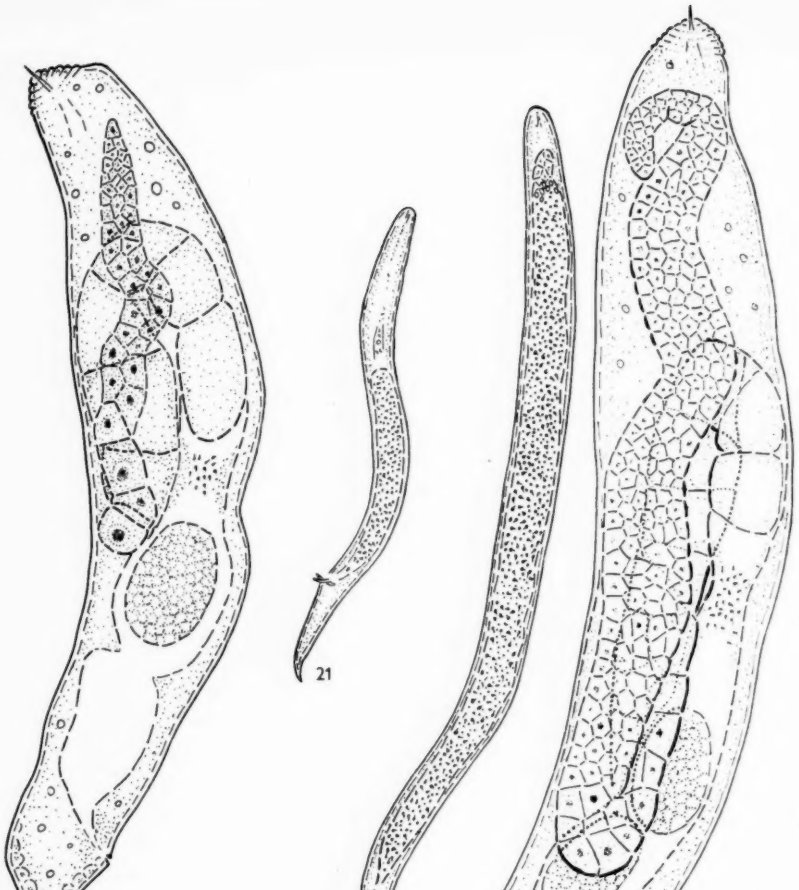
Closely resembling the female of the parasitic generation, but smaller and with much reduced gonad (Fig. 20). Preserved material usually curved slightly dorsally. Cuticle smooth, without striae, though often with transverse folds in head and tail regions. Stylet 10μ long and 0·5μ wide at base. Oesophageal and glandular structures degenerate. Gonad divided by reflexes into uterus, oviduct, and ovary. Ovary without apical reflex, extending to within 29μ of head tip. Uterus with only one or two eggs. Anus midway between vulva and tail tip. Tail containing large cells along walls.

Adult male, partially free-living generation. Type: *L* 0·139 mm.; *W* 0·008 mm.; *A* 0·020 mm.; *T* 31 %; *a* 17; *c* 6·9. Paratypes: *L* 0·137–0·166 mm., av. 0·149 mm.; *W* 0·007–0·010 mm., av. 0·008 mm.; *A* 0·017–0·023 mm., av. 0·021 mm.; *T* 31–50 %; *a* 15–23; *c* 6·7–9·1; *n* 10.

Resembling male of the parasitic generation but smaller and with reduced testis (Fig. 21). Cuticle with fine striae, especially fine on the head, where they are less than a micron wide. Lateral field commencing on head, extending to tail tip, and 2μ wide at middle of body. Short stoma and oesophagus present. Slight swelling of cuticle 36μ from tip of head suggesting an excretory pore, but no internal structure visible. Intestine indicated by vacuolate structure in front of testis. Testis filled with sperms, arising 60μ from head and extending for 54μ, occupying most of body width, and narrowing in posterior half of body. Vas deferens short. Two spicules projecting unevenly from cloaca, each gently curved, widened slightly at its base, without constriction, and tapering gradually to truncate end.

Larval stages described below.

Host and locality. The type material was found in the abdominal haemocoele of



Figs. 18–21. *Parasitylenchus diplogenus* sp.nov. 18. Adult female of the parasitic generation from haemocoele of *D. subobscura*. 19. Adult male of the parasitic generation from haemocoele of *D. subobscura*. 20. Adult female of the free-living generation from haemocoele of *D. subobscura*. 21. Adult male of the free-living generation.

a specimen of *Drosophila subobscura* Coll. collected in the Manor Woods, Rothamsted Experimental Station, Harpenden, Herts, England. The species was also found in *D. obscura* Fall., and *D. silvestris* Basden, at Harpenden, Herts, and in *D. subobscura* and *D. obscura* at Dalkeith, Scotland. Basden & Goodey (1955) also recorded it in *D. silvestris* at Dalkeith. The holotype and the allotype are in the British Museum (Natural History). Series of paratypes are in the Nematode Collection, Rothamsted Experimental Station, and the Canadian National Collection of Nematodes at Ottawa and Belleville.

Diagnosis. A species of *Parasitylenchus* that oviposits unembryonated eggs. Smaller in all generations than either *dispar* or *curvidentis*. No gubernaculum present. Spear simple. Vulva clearly visible in females of both generations. Free-living stages smaller than parasitic stages.

Older stages in both parasitic and free-living generations develop sac-like forms and lose their worm-like appearance. This change in form is accompanied by degeneration of the internal organs. The known hosts of *diplogenus* are Diptera, whereas those of *dispar* and *curvidentis* are Coleoptera, *Ips typographus* (L.) and *Pityokteines curvidens* (Germ.) respectively.

Life cycle

Parasitylenchus diplogenus has a life cycle of two sexual generations. Fuchs (1915) almost discovered this type of cycle in *P. dispar*. Though he mentioned both parasitic and free-living generations, he used 'generation' in the sense of phase, and considered that there was only one true generation, and that the large numbers of females and males in the host haemocoele entered by penetration. Even upon re-examination of the cycle in response to Wülker's (1923) queries about the presence of mature males in the host, Fuchs (1929) could not explain their origin satisfactorily. Rühm (1956) discovered the cycle for *P. curvidentis* in *Pityokteines curvidens* (Germ.), and later for *P. dispar* in *Ips typographus* (L.). In the two cycles described by Rühm the partially free-living females are larger than the females of the parasitic generation. Rühm also found ovoviviparity, whereas only oviparity occurs in *diplogenus*.

The life cycle of *P. diplogenus* starts by the penetration of an inseminated female through the cuticle and into the haemocoele of a larval host. Actual penetration of the cuticle was not observed, but seems a reasonable assumption because the eelworm has a stylet. The nematode develops in the host and at host pupation has become a gravid female. This female produces eggs that lie loose in the body cavity of the host and soon hatch into active larvae. The gravid female becomes sac-like and has no recognizable structure at the emergence of the adult fly.

The larvae develop quickly, reach maturity, and mate, and the females begin egg-laying in the host haemocoele. Dissections of host flies at this stage in the life cycle reveal gravid females, mature males, eggs and larvae. The larvae leave female hosts by descending the oviduct to the vulva. The method of exit from male hosts was not observed, but is probably through the crop and the intestine.

The nematodes drop on or near material in which *Drosophila* larvae occur. Males

mature in 4 days in tap water, but females show little genital development in this medium. Copulation takes place in culture bottles but not in water cultures. The fertilized females enter another host and commence another cycle.

The number of individuals increases strikingly with each generation. The initial infection usually involves one female; observations of the numbers in field infections ranged from one to three, whereas in laboratory infections the number was as high as eight. A single female of the first generation gives rise to ten to twenty females in the second generation. These each produce 100–200 larvae, so that the total number may be as high as 4000; 4380 were counted in one instance.

Growth and development

The adult female of the parasitic generation moults twice, as it was often found within the skins of the third and fourth larvae (Fig. 23). The male of this generation was not found in more than one skin (Fig. 24).

Two moults were found in both sexes of the free-living generation. The third moult occurs in the host gonads and the fourth while the nematodes are free-living. Nothing is known of the missing cast cuticles in either generation. Circumstantial evidence, however, suggests that a moult may occur in the egg of the free-living generation, because the well-developed embryo usually has many folds and annulations in its cuticle that resemble those of the moulting larvae.

The body lengths for a number of stages of growth in both the parasitic and the free-living generations are shown in Table 2.

Table 2. *Means and ranges of body lengths (mm.) for stages of growth of* Parasitylenchus diplogenus

(Number of specimens given in brackets.)

	Male	Female
	Parasitic generation	
Larva in host pupa	0·148 (0·133–0·166) [9]	0·146 (0·139–0·152) [4]
Third moult	0·160 (0·148–0·188) [8]	0·168 (0·160–0·173) [5]
Fourth moult	0·187 (0·170–0·203) [8]	0·199 (0·185–0·199) [5]
	Free-living generation	
Newly hatched	0·128 (0·121–0·147) [5]	0·133 (0·120–0·150) [5]
Third moult	0·156 (0·145–0·176) [6]	0·167 (0·145–0·188) [5]
Fourth moult	0·144 (0·138–0·153) [5]	—

Most growth occurs while the nematodes are parasitic; there is little in the free-living stage. This is especially true of the females of the free-living generation after their entrance into the final host.

The most striking differences in the morphology of the two generations are the smaller body length of the adults, the smaller size of the gonads, and the smaller egg production in the free-living than in the parasitic generation (Figs. 18–21). These differences are probably the result of a plentiful supply of food for the parasitic generation, as contrasted with the period of non-feeding in the free-living generation.

The species of host affects the size of the gravid female. The female parasites are larger in *D. obscura* than those in *D. subobscura* in the Scottish as well as the English material. The males show no difference in size.

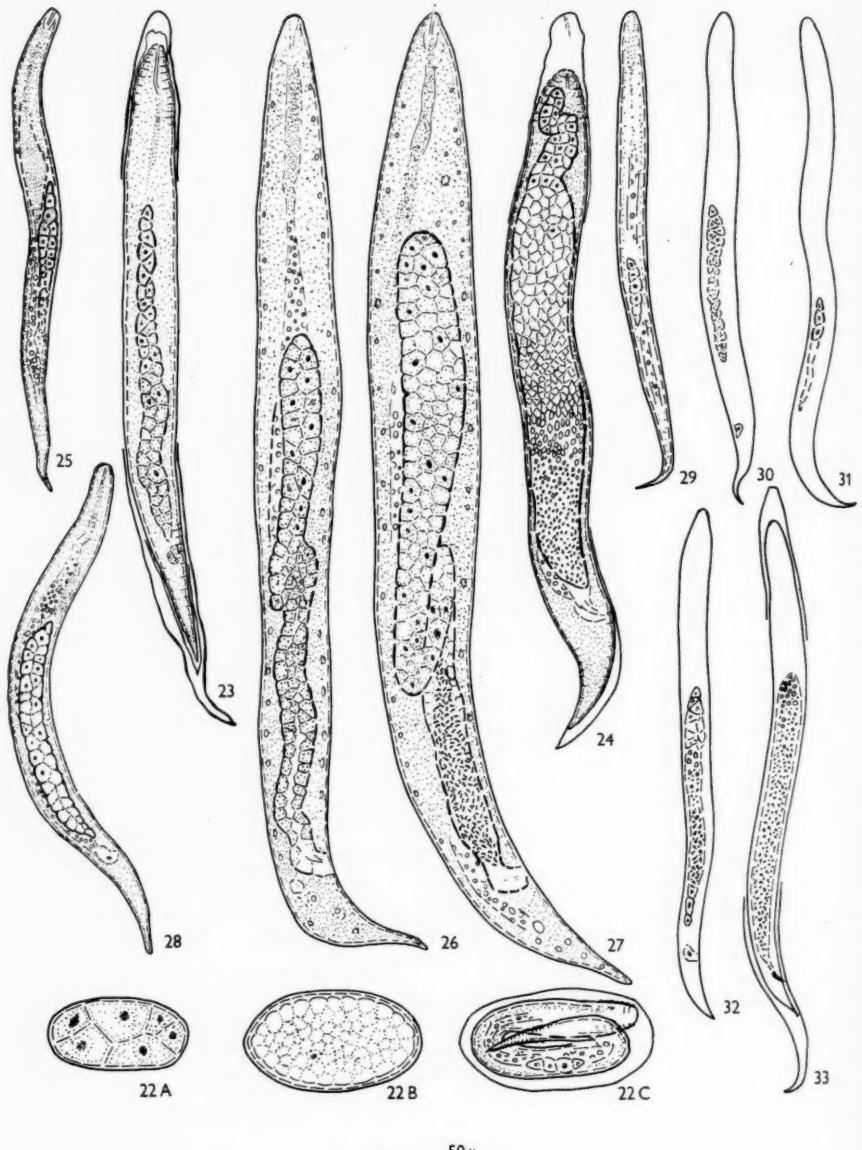
The eggs of both the parasitic and the free-living generations are ovoid at oviposition, with an undifferentiated cellular mass enclosed in a thin egg membrane, but soon they elongate and develop thicker membranes. The cell mass lengthens with a folding at one pole, followed by a second folding that divides the embryo into three sections (Fig. 22). The genital primordium of three large cells and two small terminal cells is visible in the posterior half of the body. A short length of intestine is also visible in the same region. The length of a newly hatched larva is 120–150μ.

The minuteness and lack of detail of the internal structures of this worm prevented detailed observations of its development, particularly in the free-living generation. However, the development of the genital system was traced in the parasitic generation.

The gonads of both sexes of the parasitic generation have a rapid and extensive development. In the youngest female (Fig. 25), removed from a 3-day-old pupa, the genital primordium consisted of the primordia of the ovary and the uterus. The ovarial primordium has fifteen to eighteen large cells with darkly stained nuclei. The cell structures of the uterine initial were indistinct because the cells were dividing. In adult females that are enclosed by two larval skins the ovary occupies two-thirds of the body length (Fig. 26) and consists of a single row of cells anteriorly and two or more rows posteriorly. Another mass of cellular activity in the tail indicates the primordia of the vulva and vagina. In older adults the ovary widens and swellings midway along its length are the first indications of reflexes (Fig. 27). In the inseminated female two reflexes are visible, the first indicating the beginning of the oviduct and the second the uterus (Fig. 28). The receptaculum seminis is not yet formed and, therefore, the sperms are in the uterus. In the older female the gonad has increased in length and is divided by two reflexes into the ovary, oviduct and uterus. Individual eggs first become apparent at the end of the ovary. In the oviduct the eggs are easily seen by their large nuclei and dark karyosomes. The receptaculum seminis has been formed and lies approximately at the middle of the body. Older females lose the vermiform shape and become almost rectangular in the head region upon the swelling and forward extension of the oviduct and uterus.

In young males the gonad primordium is half as long as the body (Fig. 28), and its cells are distinct with darkly stained nuclei. A spicular primordium is visible on the ventral side of the tail. In older specimens the primordia of the testis and of the vas deferens are visible. The latter has a more nearly homogeneous cell structure than the former. The testis fills most of the body of the adult male that is within the fourth larval skin (Fig. 24). The extensive growth of the testis causes it to fold in the head region. Later the spicules become visible and the body becomes almost completely occupied by the testis.

As in the parasitic generation, it was possible to determine the sex of the larval worms in the host body cavity. The genital development followed a pattern similar



Figs. 22–33. *P. diplogenus*. 22, 29–33 being of the free-living generation, and 23–28 of the parasitic generation. 22A, B, C. Various stages in development of eggs found in host haemocoele. 23. Female enclosed in cuticles of third and fourth larval stages. 24. Adult male within cuticle of fourth larval stage from haemocoele of an adult of *D. subobscura*. 25. Female larva from body cavity of pupa of *D. subobscura*. 26. Unfertilized female from haemocoele of *D. subobscura*. 27. Inseminated female from haemocoele of *D. subobscura*. 28. Early male larva from body cavity of pupa of *D. subobscura*. 29. Larva from haemocoele of *D. subobscura*. 30. Male larva from host haemocoele. 31. Female larva from host haemocoele. 32. Male larva from ovaries of *D. subobscura*. 33. Free-living adult male enclosed in the fourth larval skin.

to that of the parasitic generation (Figs. 29–33). The testis and spicular primordia are well developed when the male larva emerges from the host gonad, a few sperms being present. The female larva at this stage has a gonad composed of the ovarial and uterine primordia.

IV. INCIDENCE OF PARASITISM

A total of 7743 specimens of twelve species of *Drosophila* were collected and examined at Rothamsted. Two species were parasitized by *H. aoronymphium*, three by *P. diplogenus*, and the sixth, *D. confusa*, by an unidentified allantonematid (Table 3). The six uninfected species were: *D. deflexa* Duda, *D. funebris* (Fabr.), *D. immigrans* Sturt., *D. melanogaster* Meig., *D. tristis* Fall., and *Parascaptomyza disticha* (Duda).

Table 3. *Number of wild-caught flies and percentage infected by nematodes for six species of* Drosophila

	Uninfected		Infected			
Host species	Male	Female	Male	Female	Total	Percentage parasitism
D. kuntzei	316	449	11	23	799	4
D. phalerata	573	495	142	137	1347	20·8
D. subobscura	1547	2772	66	184	4569	5·5
D. obscura	188	371	4	7	570	2
D. silvestris	21	29	1	0	51	2
D. confusa	45	81	3	0	129	2

Collections at several localities near Dalkeith, Scotland, in November 1954, and September 1955, provided 191 specimens of five species: *D. funebris*, *D. obscura*, *D. subobscura*, *D. silvestris* and *D. tristis*. *P. diplogenus* was found in 7% of forty-three specimens of *D. obscura*, and in 3% of both the 1954 collection of seventy and the 1955 collection of thirty-four specimens of *D. subobscura*. These percentages are similar to those from Rothamsted.

The infection figures for both sexes of the three host species of *P. diplogenus* show the females to be more heavily parasitized than the males (Table 3). These figures were tested in a Contingency Table by means of a χ^2 test and only in *D. subobscura* was there a significant departure from an assumption of equal infection for each sex. Presumably a factor, or factors, favours parasitism of the female of *D. subobscura* as compared with the male.

Because *D. subobscura* adults occurred throughout the year in the field, a study of the fluctuation in the weekly parasitism by *P. diplogenus* was possible. The percentage parasitism increased during the autumn, remained static during the winter, and decreased in the spring. The numbers of *D. subobscura* caught in traps showed a similar cycle. This suggests that the incidence of parasitism of *P. diplogenus* fluctuates with the population density of the host.

Records were kept of the number of gravid females of *H. aoronymphium* found in each host during a heavy infection period in August and September 1956. Table 4 shows that one worm per fly was the most usual number with a rapid

decline for higher numbers. Two cases were found outside the observational period in which the numbers of nematodes per host were 13 and 25. All distributions showed a statistically significant departure from a Poisson distribution.

Table 4. *Number of female* Howardula aoronymphium *per fly*

Host species and sex	No. of nematodes per host						
	0	1	2	3	4	5–7	8–10
D. phalerata							
Female	287	43	25	19	7	3	2
Male	394	58	19	8	3	2	0
Total	681	101	44	27	10	5	2
D. kuntzei							
Female	307	5	4	1	0	0	1
Male	234	5	1	0	1	1	0
Total	541	10	5	1	1	1	1

V. HOST-PARASITE RELATIONS

Effect of parasite on host

No differences were found in the external morphology of parasitized and unparasitized flies, and dissection is, therefore, the only positive means of determining parasitism.

The parasites damage both the genital and the intestinal tracts, particularly when emerging from the host. The only observable effect of parasitism before emergence is the impaired development of the female gonads. Fewer eggs mature than normally, and the developing eggs and follicles are small. The fat bodies, especially those associated with the gonads, are reduced. These observations suggest that the parasites and the gonads of the host may compete for nutritive material.

The female gonads are almost completely destroyed by the emergence of the worms through the genital system. Larval worms pierce the thin peritoneal sheath surrounding the ovary and in time destroy it so that the ovarioles hang loose from their pedicels. The worms move between these loose ovarioles, pierce the epithelial sheath of the pedicels, and enter the lateral and the common oviducts. They occasionally pierce developing follicles, but rarely the basal follicles. Both the oviduct and the uterus are often tightly packed with nematodes and in a few hosts larvae were seen in the ducts that lead to the spermathecae. Where emergence is well advanced the basal follicles remain undeveloped and most of the gonad is damaged to such an extent that egg production is improbable.

The crop is damaged when the parasites leave the host by the intestinal tract. Its thin wall is soon destroyed by the penetration of the larvae on their way to the intestine. The intestine and other associated structures are little damaged. Larvae often gather in the rectum of the fly and appear to block the lumen.

Effect of host on parasite

Both *D. phalerata* and *D. kuntzei* show a reaction to *H. aoronymphium*. Enlarging parasitic females, particularly those at the hind end of the body, become covered with what appears to be fat tissue. This tissue is continuous with the fat tissue of the host organs and is supplied with minute tracheae. The worms are held fast and the tissue that surrounds older females becomes dirty yellow in colour. This does not appear to harm the nematodes as they continue to grow and develop.

Competition may occur between individual females of *H. aoronymphium* in the host haemocoele, for, as the number of gravid worms increases, the length of individual worms decreases. This competition also operates in *P. diplogenus* but to a lesser degree.

VI. SPECIFICITY OF PARASITISM

The two hosts parasitized by *H. aoronymphium*, *D. phalerata* and *D. kuntzei*, and the three parasitized by *P. diplogenus*, *D. subobscura*, *D. obscura* and *D. silvestris* have different ovipositing habits: the former oviposit on toadstools and mushrooms and the latter on tree fluxes. Because only larval hosts are parasitized and the larvae do not move from the oviposition sites, these two ovipositing habits suggest a mechanism for the restriction of the nematodes to particular hosts.

Experiments were made to determine whether *H. aoronymphium* would parasitize *D. subobscura* when the two species were reared in the same culture jar. Five other host species were tested with *aoronymphium*, and the same six hosts with *P. diplogenus*. Tests consisted of rearing together uninfected and infected hosts with suitable controls, and of discovering infection by examining the emerging adults of the filial generation.

D. phalerata and *D. kuntzei* were not infected by *P. diplogenus*, and *D. subobscura* and *D. obscura* were not infected by *H. aoronymphium*. Host specificity does not, therefore, appear to be determined by the ovipositing habits of the host, though initially this might have been a factor in the evolution of the nematode-fly relationship. This is supported by the results for *D. funebris*. This species oviposits on both tree fluxes and toadstools but was not infected by either species of nematode.

D. melanogaster is known principally from decaying fruit. It was infected by *P. diplogenus*, and this gives an indication as to the nature of the infectivity of the hosts. *D. phalerata* and *D. kuntzei* are of the subgenus *Drosophila*, and *D. subobscura*, *D. obscura*, *D. silvestris* and *D. melanogaster* are of the subgenus *Sophophora* (Sturtevant, 1942). Possibly the barrier to infectivity is correlated with characteristics that have also been used in classifying these subgenera.

VII. SUMMARY

1. Two species of Allantonematidae were found parasitizing five of twelve species of *Drosophila* collected at Rothamsted Experimental Station, Harpenden, Herts, England.

2. *H. aoronymphium* sp.nov. was found in 20·8% of *D. phalerata* and 4·3% of *D. kuntzei* examined. *Parasitylenchus diplogenus* sp.nov. was found in about 5% of *D. subobscura*, *D. obscura*, and *D. silvestris* examined.

3. The type species and taxonomy of the genus *Parasitylenchus* are discussed.

4. The life history of *H. aoronymphium* is typical of most allantonematids except that the female is fertilized when she is a fourth-stage larva. *P. diplogenus* has two sexual generations per cycle.

5. Both nematodes have a precocious development of the male and a retarded development of the female genital system. Both destroy host gonads.

6. The specificity of the nematodes is not a result of dissimilar ovipositing habits of the hosts, but shows a similarity to the taxonomic assignment of the hosts to subgenera.

SYSTEMATIC ABBREVIATIONS

L, body length. *W*, width of body at widest point. *A*, distance of anus from the tip of the tail. *V*, distance of the vulva from the head divided by the length of the worm and expressed as a percentage. *T*, the length of the testis divided by the length of the worm and expressed as a percentage. *EL*, egg length. *EW*, egg width. *a* and *c* are De Man's ratios. *n*, number of specimens.

I wish to express gratitude to the following for help and advice: Professor B. G. Peters, Imperial College of Science and Technology, London; Mr F. W. G. Jones and Dr J. B. Goodey, Rothamsted Experimental Station; and Mr E. B. Basden, Institute of Animal Genetics, Edinburgh. I am also indebted to Lawes Agricultural Trust for laboratory space at Rothamsted Experimental Station, and to the Canada Department of Agriculture for educational leave.

REFERENCES

BAKER, A. D. (1953). Rapid method for mounting nematodes in glycerine. *Canad. Ent.* **85**, 77–8.

BASDEN, E. B. (1954). The distribution and biology of Drosophilidae (Diptera) in Scotland, including a new species of *Drosophila*. *Trans. Roy. Soc. Edinb.* **62**, 603–54.

BASDEN, E. B. & GOODEY, J. B. (1955). A nematode parasite of *Drosophila*. *Nature, Lond.*, **175**, 431.

BAYLIS, H. A. & DAUBNEY, R. (1926). *A Synopsis of the Families and Genera of Nematoda*, 277 pp. London.

CHITWOOD, B. G. & CHITWOOD, M. B. (1937). *An Introduction to Nematology*. U.S.A.: Washington, D.C.

CHRISTIE, J. R. (1938). Two nematodes associated with decaying citrus fruit. *Proc. Helm. Soc. Wash.* **5**, 29–33.

COBB, N. A. (1920). One hundred new nemas. (Type species of 100 new genera.) *Contr. Sci. Nematol. (Cobb)*, **9**, 217–343.

COBB, N. A. (1921). *Howardula benigna*; a nema parasite of the cucumber beetle. *Science*, **54**, 667–70.

COURTNEY, W. D., POLLEY, D. & MILLER, V. L. (1955). TAF, an improved fixative in nematode technique. *Plant Dis. Reptr.* **39**, 570–1.

DEMEREC, M. (1948). *Biology of* Drosophila, 630 pp. New York: Wiley and Co.

FILIPJEV, I. N. (1934). [*Harmful and Useful Nematodes in Rural Economy.*] [In Russian.] 440 pp. Leningrad: Moskva.

FRANKLIN, M. T. & GOODEY, J. B. (1949). A cotton blue lactophenol technique for mounting plant-parasitic nematodes. *J. Helminth*, **23**, 175–8.

FUCHS, G. (1914). *Tylenchus dispar curvidentis* m. und *Tylenchus dispar cryphali* m. *Zool. Anz.* **45**, 195–207.

FUCHS, G. (1915). Die Naturgeschichte der Nematoden und einiger anderer Parasiten. 1. des *Ips typographus* L., 2. des *Hylobius abietis* L. *Zool. Jahrb.* (*Abt. Syst.*), **38**, 109–222.

FUCHS, G. (1929). Nachschrift zu Wülkers Bemerkungen. *Z. Parasitenk.* **2**, 291–3.

GOODEY, T. (1930). On a remarkable new nematode, *Tylenchinema oscinellae* gen. et sp.n., parasitic in the frit-fly, *Oscinella frit* L., attacking oats. *Phil. Trans.* B, **218**, 315–43.

GOODEY, T. (1931). Further observations on *Tylenchinema oscinellae* Goodey, 1930, a nematode parasite of the frit-fly. *J. Helminth*, **9**, 17–20.

VON LINSTOW, O. (1890). Über Allantonema und Diplogaster. *Zbl. Bakt.* **8**, 489–93.

MICOLETZKY, H. (1922). Die freilebende Erd-Nematoden. *Arch. Naturgesch.* (Abt. A), **87**, 1–650.

MICOLETZKY, H. (1925). Die freilebenden Süsswasser- und Moornematoden Dänemarks. *K. danske vidensk. Selsk.* 8th ser., **10**, 57–310.

OLDHAM, J. N. (1933). On *Howardula phyllotretae* n.sp., a nematode parasite of the flea beetles (Chrysomelidae, Coleoptera), with some observations on its incidence. *J. Helminth.* **11**, 119–36.

RASKI, D. J. (1950). The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. *Phytopathology*, **40**, 135–52.

RÜHM, W. (1956). Die Nematoden der Ipiden. *Parasit. SchrRheihe*, **6**, 1–437.

SCHMIDT, A. (1871). Ueber den Rübennematoden. *Z. Ver. Rüben-zuckerindustr. Zoolver.*, **121**, 1–19.

STILES, C. W. & HASSALL, A. (1920). Index catalogue of medical and veterinary zoology: subjects, roundworms (Nematoda, Gordiacea, and Acanthocephali) and the diseases they cause. *Bull. U.S. Publ. Hlth Serv., Hyg. Lab.*, **114**.

STURTEVANT, A. H. (1942). The classification of the genus *Drosophila*, with descriptions of nine new species. *Univ. Texas Publ.* **4213**, 5–51.

THORNE, G. (1935). Nemic parasites and associates of the mountain pine beetle (*Dendroctonus monticolae*) in Utah. *J. Agric. Res.* **51**, 131–44.

WACHEK, F. (1955). Die entoparasitischen Tylenchiden. *Parasit. SchrRheihe*, **3**, 1–119.

WÜLKER, G. (1923). Über Fortpflanzung und Entwicklung von *Allantonema* und verwandten Nematoden. *Ergebn. Zool.* **5**, 389–507.

(*MS. received for publication* 7. III. 1958.—Ed.)

INHIBITION OF *ENTAMOEBA HISTOLYTICA IN VITRO* BY SPECIFIC ANTIBODY*

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INTRODUCTION

Although many studies have been performed on the diagnosis of amoebiasis using the complement-fixation test, this technique is not entirely satisfactory. The lack of correlation between the complement-fixation test and clinical amoebiasis, especially amoebic dysentery, has been observed by Dolkart, Halpern & Cullin (1951), Buchman, Kullman & Margolis (1952), Paulson & Andrews (1938), Magath & Meleney (1940), and McDearman & Dunham (1952).

In an attempt to find other serological tests Greif (1947) studied agglutination of *Entamoeba histolytica* cysts with antisera. This test was of no value, since non-specific agglutination occurred with normal sera, plasma, γ-globulin, and normal horse serum. Cole & Kent (1953) described an amoeba-immobilization test designed after the *Treponema*-immobilization test. They observed that sera of rabbits immunized with *E. histolytica* had a tendency to cause motile trophozoites to cease forming pseudopods and to assume a rounded form. In preliminary studies on human sera from patients with amoebiasis, it was shown that 38 % of the sera cause immobilization of the trophozoites. Shaffer & Balsam (1954) found that *E. histolytica* antisera inhibited the ability of the amoebae to phagocytose erythrocytes *in vitro*. Goldman (1953, 1954) used the fluorescent antibody technique in studies with intestinal amoebae. *E. histolytica* antisera were reacted with a fluorescein derivative to form a protein-fluorescein conjugate. When this fluorescein antibody complex was added to the antigen (*E. histolytica* trophozoites). an antigen-antibody reaction occurred, causing the trophozoites to fluoresce when observed under the ultraviolet microscope.

Many of the serological studies in the past with *E. histolytica* have been complicated by the presence of the associated bacterial flora. Recently, Nakamura (1955) has been able to maintain the amoebae temporarily free of bacteria in a Ringer solution–coagulated egg horse serum medium fortified with the growth factors ribose-5-phosphate, adenosinetriphosphate, and sodium thioglycollate. This method made available large numbers of amoebae (approximately 1,000,000 trophozoites per ml.) free of living micro-organisms. The present study deals with the immunization of rabbits with the bacteria-free amoebae and an assay of the antisera by a culture inhibition test.

* This study was sponsored by the Commission on Enteric Infections, Armed Forces Epidemiological Board, and supported (in part) by the Office of Surgeon General, Department of the Army.

EXPERIMENTAL METHODS

The NRS, HUS–105, and UC strains of *E. histolytica* were routinely maintained in cultures and were used for immunization and assay of antibody. Bacteria-free amoebae were grown, using the method described earlier (Nakamura, 1955). The trophozoites were harvested from numerous culture tubes and frozen and thawed five to seven times in 0·4 % formalin. Merthiolate (final concentration of 1/10,000) was added as a preservative. Antigens were also prepared by desiccation, saline extraction, alcohol extraction, and grinding with alundum. However, the frozen and thawed antigens were superior to the other methods. The antigen preparations were stored in the freezer (− 24° C.) until ready for use.

White rabbits weighing 2–5 lb. were immunized by intravenous injections. This method proved superior to subcutaneous injections or subcutaneous injections with adjuvant. The marginal ear veins of the rabbit were injected; injections were given over a period of 15 days (injections being given every third day), a total of approximately 10,000,000 amoebae being injected. Seven days after the last injection, 15–25 ml. of blood were drawn from the marginal ear veins. Additional blood samples were taken from time to time for a period of several months. The sera were obtained by centrifugation of the blood after allowing the blood to clot overnight in the refrigerator. Sterilization of the sera was accomplished by filtration through a Selas porcelain microbiological filter.

The ability of *E. histolytica* antisera to inhibit growth of amoebae *in vitro* was studied in bacteria-free cultures. The antisera were added to the culture media, which consisted of a coagulated egg slant overlaid with Ringer solution, dextrose, sodium thioglycollate, rice powder (Difco), ribose-5-phosphate, adenosinetriphosphate, penicillin G (10,000 units/ml.), and streptomycin (5000 units/ml.). Experiments were performed in the presence and absence of normal horse serum; since there was no marked difference in the results, later experiments were performed in the absence of normal horse serum to eliminate possible interactions with the rabbit antisera. All tubes were sealed with a vaspar anaerobic seal after inoculating with *E. histolytica* stock cultures containing few bacterial cells (the amoebae were washed by several centrifugations in sterile saline). The overlay fluid contained the antisera in dilutions of 1/5. Control tubes contained normal rabbit sera. After inoculating the tubes with amoebae, the tubes were incubated at 37° C. for 3–4 days. Amoeba counts were made by taking approximately 0·25 ml. of the sediment from each tube, covering with a clean cover slide, and counting the number of amoebae per low-power field. Ten fields from each sample were counted and two samples were taken from each culture tube. The average counts were recorded; each serum sample was assayed in quadruplicate. The amoebae populations in media containing antisera were compared with amoebae populations in media containing normal rabbit sera.

RESULTS

Incorporation of *E. histolytica* antisera into bacteria-free amoeba cultures resulted in a decrease in total amoeba populations. Greatest inhibition *in vitro* was obtained

by rabbit sera obtained by intravenous immunization. Sera from human subjects with past histories of amoebic infections reduced the total amoeba count per low-power field, but the extent of inhibition was not too great. In no case did amoeba antisera cause complete inhibition of the amoeba in cultures. The results are tabulated in Table 1. Rabbit antisera produced 31·1–67·6 % inhibition of the

Table 1. *Inhibition of bacteria-free* Entamoeba histolytica *cultures with antisera*

Sera added to culture medium	Amoeba count per low-power field (average of four determinations)	Percentage inhibition of control
Normal rabbit serum	179	0
Rabbit antiserum (immunized by intravenous injections)	58	67·6
Rabbit antiserum (immunized by subcutaneous injections)	124	31·1
Rabbit antiserum (immunized by subcutaneous injections with adjuvant)	102	43·1
Human serum (D.K.)	72	59·8
Human serum (H.G.)	116	35·2
Human serum (R.H.)	145	19·0
Human serum (C.R.)	90	49·8

control. Human serum D.K. (male medical student) produced the greatest inhibition of the amoeba cultures as compared with other human sera. Human serum H.G. was obtained from a male student who had undergone chemotherapy for intestinal amoebiasis 4 months prior to this serological assay. Serum H.G. produced a greater inhibition than human serum R.H. which was obtained from a male student who had amoebic dysentery 2 years prior to this examination. Human serum C.R., from a subject who had been operated upon for the removal of an amoebic granuloma, produced 49·8 % inhibition of the control.

Surprisingly, there was little or no difference in the reactions of the different strains of *E. histolytica* studied. Antisera to strain NRS produced inhibition of cultures of NRS, UC, and HUS-100 strains; antisera to strain UC produced inhibition of NRS, UC and HUS-100 cultures; and antisera to strain HUS-100 produced inhibition of all three strains.

DISCUSSION

Since specific antisera inhibited *E. histolytica in vitro*, it is highly probable that the antisera contain a component which inhibits amoebic growth. This substance may be identical with the component in antisera which immobilize the trophozoites as described by Cole & Kent (1953) and with the component in antisera which lower the phagocytic activity of amoebae (Shaffer & Balsam, 1954). It is not yet clear, from the present study, whether the culture-inhibiting antibodies actually possess an amoebocidal or amoebostatic activity or merely interfere with the metabolic and growth processes of the amoebae. The fact that complete inhibition was not obtained suggests that perhaps the amoebae are able to overcome slowly

the inhibitory action of the antibodies. Cole & Kent reported that immobilized trophozoites gradually regained their motility. One disadvantage of this assay method for antibodies is that the maximum amount of the antisera which can be tested is a dilution of 1/5, since this is the optimal serum-Ringer solution ratio for the growth of amoebae. It is possible that, if normal horse serum is added, additional dilutions can be made without affecting the nutritional requirements of the amoebae. A further disadvantage is that considerable volumes of antisera are required for a complete assay, whereas the agar gel double diffusion technique for detecting *E. histolytica* antisera reported by Nakamura & Baker (1957) requires only minute amounts of sera.

Additional human sera must be assayed before the usefulness of this method can be fully evaluated with regard to its value in serodiagnosis. Also, numerous strains of *E. histolytica* must be evaluated by this technique to determine the possibilities of cross-reactions occurring and also to learn more about the serotypes of this pathogenic amoebae.

SUMMARY

Bacteria-free cultures of *E. histolytica* have been inhibited by specific and related antibodies in the antisera produced in rabbits. Maximal culture-inhibiting antibodies were produced when the rabbits were immunized with frozen and thawed, formalin-treated trophozoites injected intravenously. Human sera from subjects with current or past histories of amoebiasis also inhibited growth of amoebae *in vitro*.

REFERENCES

Buchman, E., Kullman, H. J. & Margonis, G. F. (1952). Evaluation of the complement-fixation test for amebiasis. *Gastroenterology*, **21**, 391.

Cole, B. A. & Kent, J. F. (1953). Immobilization of *Endamoeba histolytica in vitro* by antiserum produced in the rabbit. *Proc. Soc. Exp. Biol., N.Y.*, **83**, 811.

Dolkart, R. E., Halpern, B. & Cullen, J. (1951). The diagnosis of amebiasis, the role of the complement-fixation test, and the incidence of the disease in the Chicago area. *J. Lab. Clin. Med.* **38**, 804.

Greif, R. L. (1947). Agglutination of *Endamoeba histolytica* cysts. *Amer. J. Trop. med.* **27**, 131.

Goldman, M. (1953). Cytochemical differentiation of *Endamoeba histolytica* and *Endamoeba coli* by means of fluorescent antibody. *Amer. J. Hyg.* **58**, 319.

Goldman, M. (1954). Use of fluorescein-tagged antibody to identify cultures of *Endamoeba histolytica* and *Endamoeba coli*. *Amer. J. Hyg.* **59**, 318.

Magath, T. B. & Meleney, H. E. (1940). Complement fixation reaction for amebiasis; comparative tests performed by two laboratories. *Amer. J. Trop. Med.* **20**, 211.

McDearman, S. C. & Dunham, W. B. (1952). Complement-fixation tests as an aid in the differential diagnosis of extra-intestinal amebiasis. *Amer. J. Trop. Med. Hyg.* **1**, 182.

Nakamura, M. (1955). Growth factors for *Endamoeba histolytica*. *Proc. Soc. Exp. Biol., N.Y.*, **89**, 680.

Nakamura, M. & Baker, E. E. (1957). Agar diffusion precipitin technic for the detection of antibodies against *Endamoeba histolytica*. *Bact. Proc.* p. 95.

Paulson, M. & Andrews, J. (1938). Complement fixation in amebiasis. A comparative evaluation in clinical practice. *Arch. Int. Med.* **62**, 562.

Shaffer, J. G. & Balsam, T. (1954). Ability of *Endamoeba histolytica* to phagocytose red blood cells. *Proc. Soc. Exp. Biol., N.Y.*, **85**, 21.

(*MS. received for publication* 19. III. 1958.—Ed.)

IXODES TATEI N.SP. FROM IRAQ (ACARINA: IXODIDAE)

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(With 7 Figures in the Text)

The new species described here, found in the collections of the Chicago Natural History Museum, was forwarded to me by Harry Hoogstraal, NAMRU-3, Cairo, Egypt, for description. All measurements are given in millimetres.

Ixodes tatei n.sp.

Holotype. Female (Figs. 1, 2) partly fed, length 6·0, greatest breadth 4·0, Kirkuk Liwa, Chemchemal Valley, Jarmo, Iraq, 7 April 1935. C. A. Reed *leg.* Host: *Vulpes vulpes* CR 408, Cat. no. 84472. Deposited in the Chicago Natural History Museum.

Paratypes. Two females, well fed: dimensions 7×5 and $7{\cdot}5 \times 4{\cdot}9$, Etbil Liwa, Zagros Mts., Iraq, 2 November 1952. Host: 'Rock Partridge'. Deposited in the Chicago Natural History Museum.

Description. Colour of capitulum and legs dark brown, scutum paler. Post-scutal area and venter with few fine hairs.

Capitulum (Fig. 3). Length, tips of palpi to tips of cornua 0·85; greatest width of basis, 0·5. Dorsal surface of basis smooth, shining. Porose areas large, piriform, depressed and separated from one another by a narrow interval. Cornua short, pointed, but nevertheless distinct. *Palpi* widest near the apex of article 2; outer margin of segment 2 slightly concave, that of article 3 almost straight; distally on segment 2, and proximally on segment 3, an elongate oval depression (shaded in Fig. 3). Combined length of segments 2 and 3 about 0·58. In ventral view (Fig. 4) the basis is flattened, only slightly constricted in the middle, postero-lateral angles rounded, posterior margin nearly straight. Auriculae as distinct short, strong, tapering spurs. Transverse suture line present. Palpal segment 1 with distinct ventral plate.

Hypostome. Broken in all three specimens, but as far as can be ascertained the dentition behind an apical corona as 3 files of 4/4, 3 files of 3/3, 3 files of 2/2. Teeth of the lateral files largest. Length about 0·5.

Scutum (Fig. 5). Length 1·34; width 0·98. Shape as figured. Lateral carinae lacking. Cervical grooves as shallow divergent linear depressions, with slight straight ridge lateral to the grooves. Punctations numerous, moderate to large in size, larger in antero-lateral field, deep. Hairs in paratypes very long and abundant.

Legs (Fig. 6). Coxa I with broad internal spur of moderate length, external spur broader basally than long; coxa II with broad rounded external spur, internal angle as a marginal salience; coxa III with similar but smaller external spur, which on IV is reduced to a weak tuberosity. Trochanteral spurs absent. Tarsi

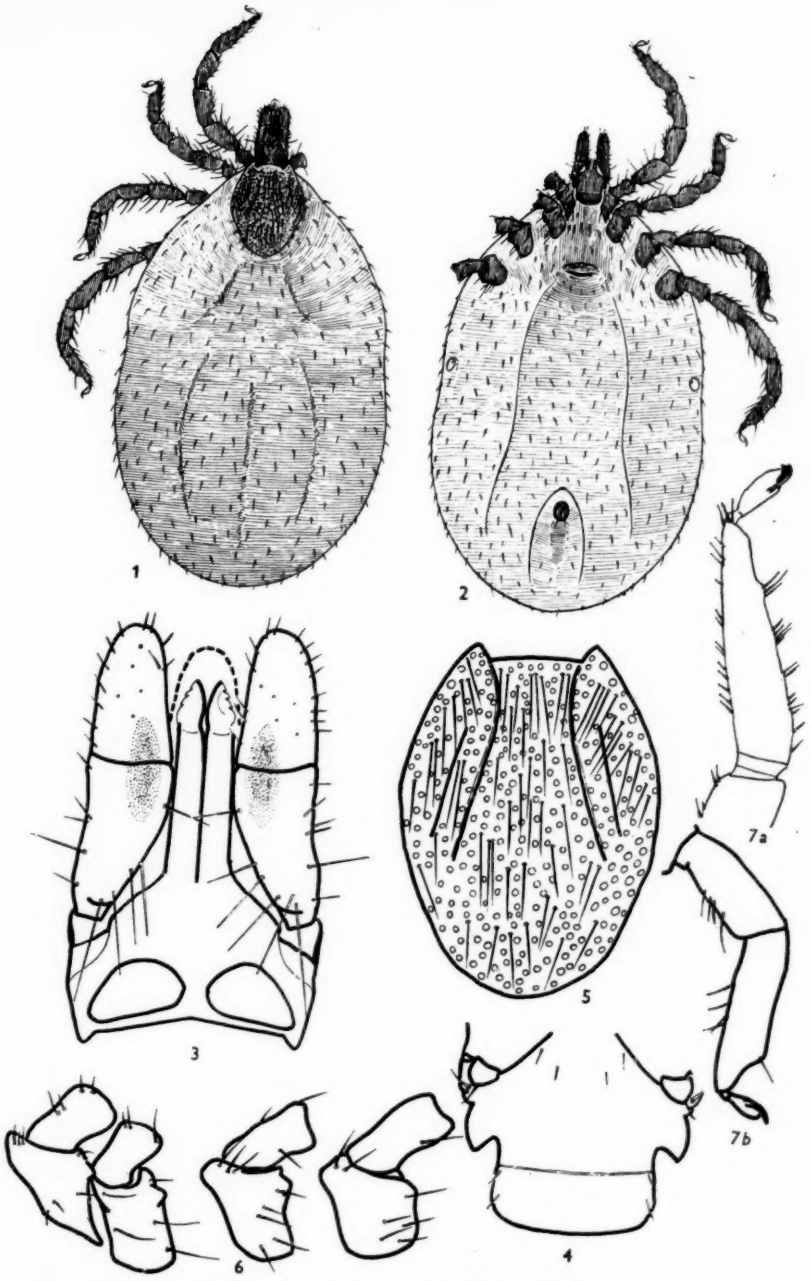

Fig. 1. Holotype female, dorsal view.
Fig. 2. Holotype female, ventral view.
Fig. 3. Capitulum, dorsal.
Fig. 4. Basis capituli, ventral.
Fig. 5. Scutum.
Fig. 6. Coxae and trochanters.
Fig. 7. *a*, tarsus I; *b*, tarsus IV.

(Fig. 7*a*, *b*) gradually narrowing subterminally, length of tarsus I, 0·82; metatarsus I, 0·47; length of tarsus IV, 0·67; metatarsus IV, 0·50.

Spiracular plate. Transversely oval, goblets of moderate size and number. Pale in colour. Dimensions 0·32 × 0·24.

Genital aperture. On a level with the third intercoxal space. Male and immature stages not known.

Hosts. Vulpes vulpes and 'rock partridges'.

Related species. This species has affinities with *Ixodes redikorzevi redikorzevi* Olenev 1927, and the *I. laguri* group of species. According to Pomerantzev (1950), *I. laguri* Ol. is separated from *I. redikorzevi* by the development of membranous extensions on the posterior borders of coxae I and II; in the former such extensions are well marked, but in the latter they are lacking. Similarly, in *I. tatei* n.sp. these membranes are lacking so that it should fall into the group of *I. redikorzevi*. The coxal pattern, however, does not support this contention. In *I. redikorzevi* the internal spur on coxa I of the female is long, narrow, tapering and overlaps the front edge of coxa II (Arthur, 1955, fig. 6; Pomerantzev, 1950, fig. 149) with the external spur just over half as long; the external spurs on coxae II and III are as long or longer than their basal widths. In *I. tatei* the internal spur on coxa I is of moderate length, very broad basally and does not overlap coxa II; the external spur is short and much broader basally than it is long, and similar conditions apply to coxae II and III, whilst the external spur of coxa IV is reduced to a mere protuberance. Apart from the marginal outgrowths, *I. tatei* n.sp. can be recognized from the *I. laguri* group by the use of the following key.

Scutum oblong oval, hairs sparse.		***I. laguri laguri*** Ol. 1929.
Scutum ovate or long and narrow; hairs sparse to hairs abundant.		2
2	Internal spur on coxa I parallel sided; hairs sparse on scutum.	3
	Internal spur of coxa I drawn out into a broad triangular prolongation. Hairs very abundant on scutum.	***I. tatei*** n.sp.
3	Scutum ovate; even punctations.	***I. laguri armeniacus*** Pom & Kirsch. 1946
	Scutum long and narrow, punctations vague	***I. laguri colchicus*** Pom. 1946

The species is named for Dr P. Tate, Director of the Molteno Institute of Parasitology, Cambridge, for his very kind assistance at all times.

REFERENCES

ARTHUR, D. R. (1955). *Ixodes redikorzevi redikorzevi* Ol. (Ixodoidea, Ixodidae) in Egypt, with a consideration of its synonymy. *J. Egypt. Publ. Hlth Ass.* **30**, 39–56.

POMERANTZEV, B. I. (1950). *Fauna of U.S.S.R. Arachnida*, **4**, No. 2, 224 pp. Acad. U.S.S.R., Moscow, Leningrad.

(*MS. received for publication* 4. XI. 1957.—Ed.)

NEODIPLOSTOMUM INTERMEDIUM N.SP. FROM THE ALLIED RAT, *RATTUS ASSIMILIS*, WITH REMARKS ON THE GENERA *NEODIPLOSTOMUM* AND *FIBRICOLA* (TREMATODA: DIPLOSTOMATIDAE)

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(With 8 figures in the Text)

INTRODUCTION

In a survey of the helminth parasites of native rats, 61 mature specimens of a diplostome trematode were found in the small intestine of one of 26 allied rats *Rattus assimilis*, collected during 1956 and 1957 at Mt Glorious, Queensland. This parasite was not found in two eastern swamp rats, *R. lutreolus*, and its eggs were not found in the faeces of 23 *R. assimilis*, four *R. lutreolus* and 10 tree rats, *Melomys cervinipes*, from the same locality which were held in captivity. As this diplostome appears to be new, the name, *Neodiplostomum intermedium* n.sp. is proposed for it.

DESCRIPTION

The description is based on the study of dorsal, ventral, and lateral views of whole mounts, and of sagittal serial sections. Measurements are given in the text for the type specimen; in addition the average and range of measurements for the type and nine paratypes are given in Table 1.

Diagnosis (Fig. 1). Diplostomatidae. Small worm 1·13 mm. long. Bisegmentation well-marked; forebody oval, foliaceous, 670 μ long by 360 μ wide, tapering anteriorly and without shoulders on the inrolled margin, spiny on ventral surface; hindbody cylindrical, not flexed dorsally, shorter and narrower than forebody, 460 μ long by 220 μ wide, without spines. Oral sucker terminal, but opening ventrally, 59 μ long by 45 μ wide; ventral sucker 270 μ from anterior end, 62 μ long by 57 μ wide. Holdfast elliptic, 170 μ long by 120 μ wide, with anterior border 335 μ from anterior end, and 12 μ from ventral sucker, with median, longitudinal, ventral slit, and containing holdfast gland postero-dorsally.

Prepharynx not apparent (but seen in sagittal sections); pharynx 40 μ long by 28 μ wide; oesophagus short, 50 μ long; distance from bifurcation of gut to anterior border of ventral sucker 120 μ; caeca do not enter holdfast and extend in hindbody to, or slightly beyond, posterior edge of posterior testis.

Ovary triangular, submedian on right side, situated just behind junction of fore- and hindbody, 64 μ long by 83 μ wide; anterior testis subrectangular 107 μ long by 160 μ wide, almost as wide as posterior testis, cut away on left side posteriorly to accommodate Mehlis's gland; posterior testis 109 μ long by 164 μ wide, subrectangular, concave on posterior face next to coiled seminal vesicle. Vitelline follicles large, largest in holdfast and hindbody, attaining 57 × 36 μ in latter; abundant in forebody, extending anteriorly just beyond ventral sucker; reduced in hindbody to two ventro-lateral, single-file bands of large follicles which extend to posterior border of the seminal vesicle; vitelline reservoir inter-testicular, on right side. Uterus does not enter forebody, contains seven eggs. Genital bursa small, shallow, subterminal, opens dorsally, lacks a genital cone; hermaphroditic canal opens by a transverse slit in ventral wall of bursa.

* Senior Research Fellow in Helminthology.

Host. Allied rat, *Rattus assimilis* Gould.
Location. Small intestine.
Locality. On the edge of rainforest, Mt Glorious, Queensland, Australia.
Specimens. Type and 3 paratypes: U.S. Nat. Mus. Helm. Coll. nos. 38,276 and 38,277. Paratypes: South Australian Museum, Adelaide, no. E. 695–6–7; coll. Dubois (Neuchâtel); and author's collection.

Table 1. *Measurements of type material*, Neodiplostomum intermedium

(Average, and range, of type and 9 paratypes)

Total length	1·13 (1·05–1·25) mm.	Ratio holdfast length/forebody length	1/3·5–1/4·4
Forebody length	0·68 (0·63–0·78) mm.	Dist. ph. to bifurc. of gut	54 (50–81) μ
Forebody width	0·37 (0·32–0·41) mm.	Dist. bifurc. to v.s.	122 (111–137) μ
Hindbody length	0·45 (0·42–0·47) mm.	Egg	66–76 × 49–52 μ
Hindbody width	0·23 (0·22–0·26) mm.	Ovary length	60 (55–66) μ
Ratio hindbody length/forebody length	0·65 (0·60–0·70) mm.	Ovary width	84 (76–95) μ
Oral sucker length	52 (43–59) μ	Ant. testis length	110 (88–120) μ
Oral sucker width	48 (44–50) μ	Ant. testis width	160 (150–180) μ
Pharynx length	41 (38–45) μ	Post. testis length	110 (100–120) μ
Pharynx width	30 (27–32) μ	Post. testis width	170 (150–200) μ
Ventral sucker length	55 (50–57) μ	V.s. to holdfast	ca. 10 μ
Ventral sucker width	60 (59–66) μ	V.s. to flap over holdfast	50–80 μ
Holdfast length	190 (150–210) μ		
Holdfast width	71 (64–85) μ		

Appearance of holdfast. In the type specimen (Fig. 1) the holdfast is protracted, and projects beyond the level of the ventral surface of the forebody; it is broadly elliptical being 170 μ long by 120 μ wide, and contains numerous large vitelline follicles. In the paratypes, however, the holdfast is retracted. In this condition (Fig. 2) it appears as an elongate oval body 150–210 μ long by 64–85 μ wide, with a median longitudinal sulcus, lying flush with the ventral surface of the forebody and with its anterior end covered by a thin fold of the forebody extending back for 52–78 μ from the ventral sucker. The large vitelline follicles, which are within the protracted holdfast, appear to be separate from the retracted holdfast and are arranged in a single row on each side. The same condition, with holdfast retracted and covered anteriorly by a flap, was described by Pearson (1956) for the diplostomulum of *Alaria arisaemoides*.

The difference in appearance, shape, and size of retracted and protracted holdfasts is so striking that it could possibly lead to errors in identification. This apparently happened in the case of *Fibricola laruei* Miller, 1940. This species was separated from *F. cratera* (Barker & Noll) Dubois, 1932, largely by the small size and oval shape of the holdfast organ. It was later reduced to a synonym of *F. cratera* by Read (1948). A comparison of the figures of *F. cratera* of Dubois (1938, p. 358, fig. 250) and Barker (1915, pl. 2, fig. 1) with that of Miller (1940, fig. 1) suggests that the holdfast was protracted in the specimens studied by Barker & Noll and by Dubois, but was retracted in those examined by Miller.

Spination. While spines were difficult to see on whole mounts, it could be seen from serial sections that the whole ventral surface of the forebody is densely covered with fine spines which project beyond the outer border of the cuticle. The

cuticle covering the ventral surface of the forebody is thinner than that over the rest of the body. The dorsal surface of the forebody also bears numerous fine spines, but these are completely embedded in the cuticle. The hindbody is spineless.

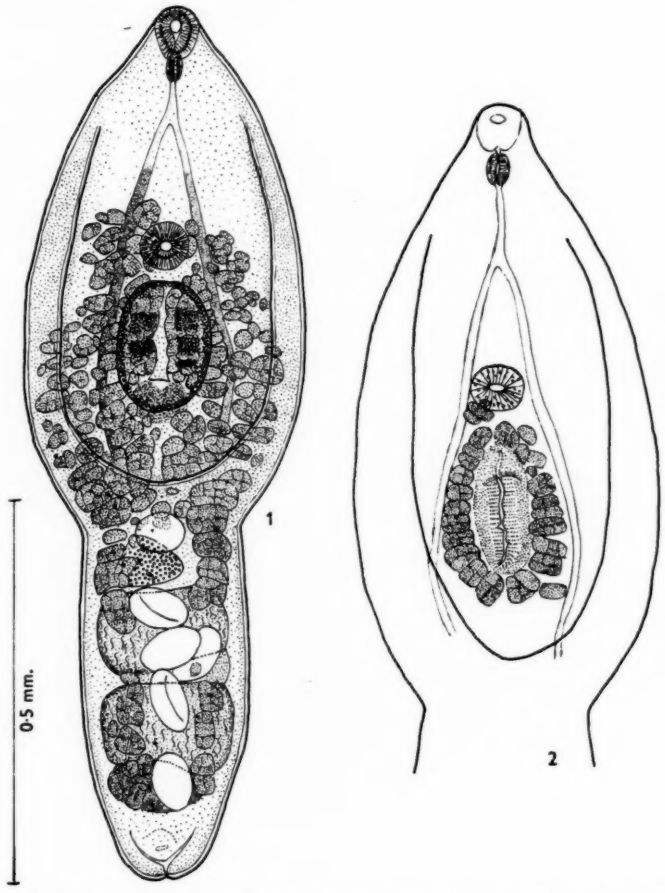


Fig. 1. *Neodiplostomum intermedium*, type specimen (ventral view; camera lucida).
Fig. 2. Ventral view of a paratype with holdfast retracted.

Reproductive system (Fig. 3), amphitypic

Female. The triangular ovary is submedian and lies just behind the junction of the fore- and hindbody. The oviduct arises postero-dorsally in the midline, shortly giving rise to Laurer's canal, which opens dorsally in the midline. The oviduct then runs laterad and ventrad passing back lateral to the anterior testis to Mehlis's gland, where it is joined by the vitelline duct from the vitelline reservoir. Ootype and Mehlis's gland lie more or less between the testes on the side opposite that to which the ovary is displaced. The uterus runs postero-dorsad to the inter-testicular zone, where it turns ventrad and mediad, and then comes forward ventral to the

anterior testis as the ascending limb of the uterus. This limb turns back upon itself at the junction of the fore- and hindbody and, as the descending limb, runs back ventrally in the midline, to the genital bursa.

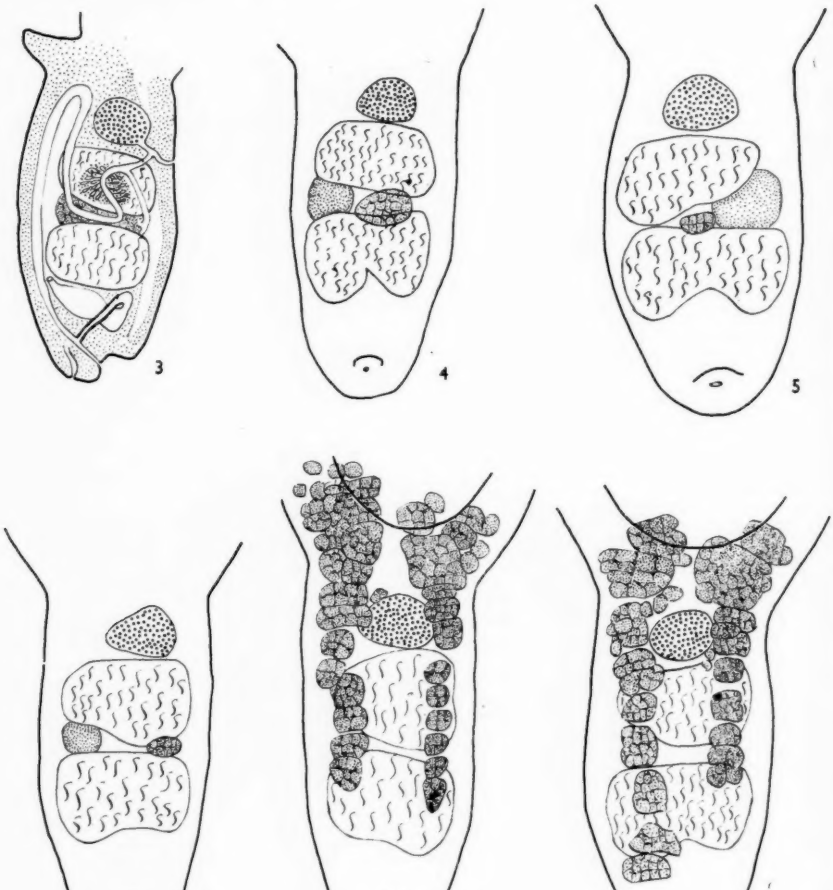


Fig. 3. Reconstruction of reproductive system from sagittal sections.

Figs. 4–6. Camera lucida outlines of hindbody of paratypes showing variation in shape of testes (dorsal views).

Figs. 7, 8. Camera lucida outlines of hindbody of paratypes showing variation in extent of vitellaria (ventral views).

The vitelline follicles are unusually large and discrete, especially in the hindbody. In the forebody they are densest beside and behind the holdfast; they extend anteriorly to, or just beyond, the anterior margin of the ventral sucker. In the hindbody the large follicles form two distinct lateral bands on the ventral side

which extend at least as far as the mid-point of the posterior testis (Fig. 7), and usually extend beyond the posterior border of the posterior testis (Fig. 1). In five of the nine paratypes the band of vitellaria on the same side as the vitelline reservoir is shorter than the other band (Fig. 8). The vitelline reservoir lies between the testes, opposite Mehlis's gland.

Male. The anterior testis is as wide, or almost as wide, as the posterior testis; it is almost symmetric in some specimens (Fig. 4), but is subtriangular in others (Figs. 5, 6). The posterior testis varies in shape from subrectangular (Fig. 6) to bilobed posteriorly (Figs. 4, 5). Both testes have a median longitudinal sulcus on the ventral face to accommodate the vas deferens. Vasa efferentia arise antero-ventrally and join near the anterior border of anterior testis to form the vas deferens, which is thin-walled, large, and full of spermatozoa. The vas deferens loops back upon itself anterior to the anterior testis and then runs posterad, ventral to the testes and dorsal to the uterus, to the posterior border of the posterior testis where it turns dorsad and dilates to become the seminal vesicle. The seminal vesicle is sinuous, and gives rise to the ejaculatory duct dorsally. The ejaculatory duct is slender, thick-walled, and runs postero-ventrad to join the uterus. No trace of a periprostate was seen. The hermaphroditic canal, which is formed by the union of uterus and ejaculatory duct, is short and opens directly into the genital bursa by a transverse slit. Genital cone absent.

The morphology of the reproductive system agrees generally with that of *F. cratera* as described by Ulmer (1955), except that the ejaculatory duct is relatively longer and apparently more muscular in *Neodiplostomum intermedium*.

Comparison

N. intermedium shows affinities with species of both *Neodiplostomum* and *Fibricola*. It resembles *Fibricola* spp. in having the vitellaria reduced in the hindbody and in parasitizing a mammal, but differs in having a nearly symmetric anterior testis, elongate holdfast, and well marked bisegmentation. *N. intermedium* resembles species of *Neodiplostomum* (*Conodiplostomum*) in having a nearly symmetric anterior testis, but differs in lacking a genital cone. *N. intermedium* differs in having a nearly symmetric anterior testis from all of the species of *Neodiplostomum* (*Neodiplostomum*) listed by Dubois (1953), except *N. cochleare* (Krause) La Rue, 1926, *N. conicum* Dubois, 1937, and *N. americanum* (Chandler & Rausch) Dubois & Rausch, 1948. *N. intermedium* differs from these three species in having the vitellaria in two ventro-lateral bands in the hindbody. Since Dubois (1953), three species of *Neodiplostomum* (*Neodiplostomum*) have been described by Bisseru (1956) from African birds of prey, namely *N. berghaani*, *N. prudhoei* and *N. pseudogypsis*. These differ from *N. intermedium* in having an asymmetric anterior testis, and vitellaria not in two simple bands. Of the species of *Neodiplostomum* not assigned to subgenera by Dubois (1953), *N. intermedium* resembles *N. gumbudia* Gogate, 1940, in having a nearly symmetric anterior testis, but differs according to the key in Dubois (1953) in having an oval holdfast and a forebody which is narrower than wide.

Generic determination

The limits of the closely related genera *Neodiplostomum* and *Fibricola* have been subject to some controversy. As defined by Dubois (1932, 1938), the genus *Fibricola* Dubois, 1932, differed from the genus *Neodiplostomum* Railliet, 1919, in having the vitelline follicles confined to the forebody and in being parasitic in mammals rather than birds. At this time (1932), Dubois placed *Neodiplostomum* and *Fibricola* in a new subfamily, Neodiplostominae, but later (1936) he reduced this subfamily to a synonym of the subfamily Diplostominae and put *Neodiplostomum* in the Diplostominae and *Fibricola* in the Alariinae. Later, Chandler (1942) described *F. texensis* in which the vitelline follicles extended to a variable extent into the hindbody. Chandler realized that *F. texensis* was intermediate in form between *N. lucidum* La Rue & Bosma, 1927, and *Fibricola* spp., but placed it in the latter genus because *Fibricola* had fewer species. This is shown by his remark that: 'If it were not for the fact that the genus *Neodiplostomum* is already large and unwieldy there would be justification for merging the genus *Fibricola* with it.' Dubois (1944) erected the genus *Theriodiplostomum* (subfamily Diplostominae) for *N. lucidum* and *F. texensis*, largely because they occur in mammals (character of *Fibricola* and hence of subfamily Alariinae) and the vitelline follicles extend into the hindbody (character of *Neodiplostomum* and hence of subfamily Diplostominae). He considered (p. 88) *Theriodiplostomum* to be intermediate between *Neodiplostomum* and *Fibricola*. Chandler & Rausch (1946) and Read (1948) felt that there was no justification for the erection of the genus *Theriodiplostomum* or for the separation of *Fibricola* and *Neodiplostomum* in separate subfamilies. Read considered that the only reason for retaining *Fibricola* separate from *Neodiplostomum* is the tendency toward concentration of the vitellaria in the forebody in *Fibricola*. Dubois & Rausch (1950) agreed with Read and suppressed *Theriodiplostomum* as a synonym of *Fibricola*. They redefined *Fibricola* and separated it from *Neodiplostomum* on the basis of the tendency toward the concentration of vitellaria in the forebody (reduction in number and density, but increase in size of follicles) and the adaptation to mammals. On this basis, *N. intermedium* appears to belong to *Fibricola*, since it is parasitic in a mammal and has the vitellaria reduced (to two bands) in the hindbody. Nevertheless, when other characters, such as the median or submedian ovary, almost symmetric anterior testis, elongate holdfast, and well-marked bisegmentation of the body, are considered, this species clearly belongs in *Neodiplostomum*.

Relationships

The subgenus *Conodiplostomum* Dubois, 1937, is characterized (Dubois, 1953) by the possession of a genital cone and, with a single exception, a symmetric anterior testis; the subgenus *Neodiplostomum* Dubois, 1937, is characterized by the absence of a genital cone and, with three exceptions, the possession of an asymmetric anterior testis. If the subgenus *Neodiplostomum* is derived from the subgenus *Conodiplostomum*, as Dubois (1944) has suggested, then *N. intermedium*, with almost symmetric anterior testis, stands near the origin of the subgenus *Neodiplo-*

stomum, and shows affinities with *N. conicum*, *N. cochleare* and *N. americanum*, parasitic in owls. On the other hand, *N. intermedium* shows affinities with *Fibricola* spp. of Dubois (1953) in its occurrence in a mammal and in the reduction of the vitellaria in the hindbody. *N. intermedium* appears to be intermediate between *F. lucida* and the species of *Neodiplostomum* (*Neodiplostomum*) listed above, and to form another link in the phylogenetic series proposed by Chandler (1942) and Dubois (1944), linking these two genera (Diagram I). The relationships of *N. intermedium* which are suggested by its affinities are shown diagrammatically below (Diagram II). In preparing this diagram of relationship the assumption is made that all of the species parasitic in mammals are derived from a single line of evolution

Diagram I. *Earlier conception of relationship of* Fibricola *and* Neodiplostomum

(After Chandler, 1942; Dubois, 1944.)

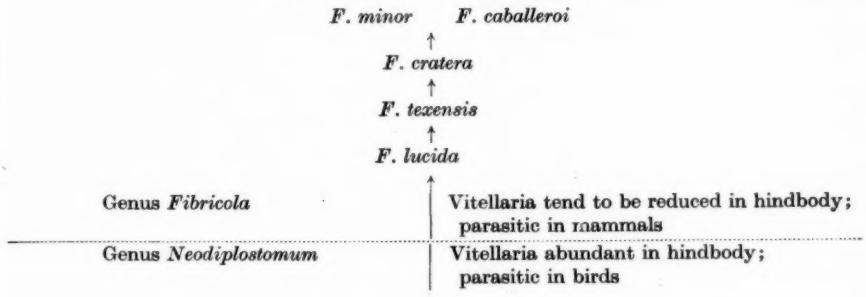
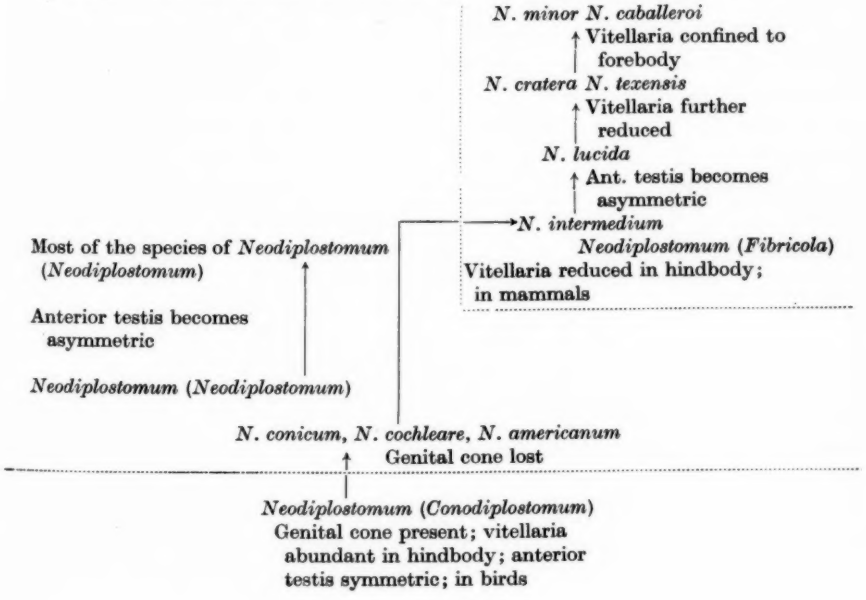


Diagram II. *Proposed relationship of* Fibricola *and* Neodiplostomum


N. minor N. caballeroi
Vitellaria confined to forebody
N. cratera N. texensis
Vitellaria further reduced
N. lucida
Ant. testis becomes asymmetric
N. intermedium
Neodiplostomum (Fibricola)
Vitellaria reduced in hindbody; in mammals
Most of the species of Neodiplostomum (Neodiplostomum)
Anterior testis becomes asymmetric
Neodiplostomum (Neodiplostomum)
N. conicum, N. cochleare, N. americanum
Genital cone lost
Neodiplostomum (Conodiplostomum)
Genital cone present; vitellaria abundant in hindbody; anterior testis symmetric; in birds

from the subgenus *Neodiplostomum* (*Neodiplostomum*). If the scheme given in Diagram II is essentially true, then it follows that the asymmetry of the anterior testis in *Fibricola* spp. and in most *Neodiplostomum* spp. is a parallel development. The validity of the distinction between *Fibricola* and *Neodiplostomum*, which was doubted by Chandler (1942) and questioned by Chandler & Rausch (1946), but supported by Read (1948) and by Dubois & Rausch (1950), must again be questioned since the form described here is intermediate between the genera *Neodiplostomum* and *Fibricola* emend. Dubois & Rausch, 1950. Two solutions to this problem suggest themselves: (*a*) that *Fibricola* be reduced to synonymy with *Neodiplostomum* (*Neodiplostomum*) since there is a series of intermediate forms, or (*b*) that *Fibricola* be made a subgenus of *Neodiplostomum*. The second solution appears to be better because it would suggest that there are three equal and closely related groups, *Conodiplostomum*, *Neodiplostomum* and *Fibricola*. Also, putting the species described here with the other species of *Fibricola* would unite in one group those species characterized by a tendency toward reduction of vitellaria in the hindbody and by occurrence in mammals. These two characters are considered by Dubois (1944) and Dubois & Rausch (1950) to have been of prime importance in the phylogeny of *Fibricola* spp. If *Fibricola* is to become a subgenus of *Neodiplostomum*, then the subfamily Alariinae must fall as a synonym of the subfamily Diplostominae as the difference in distribution of vitellaria no longer holds. Further the subfamily Alariinae, as defined by Dubois (1938, 1953), comprises genera with very different features, such as those without lappets (or pseudosuckers) or genital cone (*Fibricola*, *Pharyngostomum*, *Podospathalium*), those with lappets but without genital cone (*Didelphodiplostomum*, *Enhydridodiplostomum*), and those with both lappets and genital cone (*Alaria*, *Pharyngostomoides*).

It is proposed that the genus *Fibricola* be made a subgenus of the genus *Neodiplostomum*, and that the species, *N. intermedium* described here be placed in the subgenus *Fibricola*. In order to accommodate *N. intermedium*, the emended definition of *Fibricola* by Dubois & Rausch (1950) must be further emended to read: ovary median, submedian, or lateral, and anterior testis asymmetric or nearly symmetric. The species described herein becomes *Neodiplostomum* (*Fibricola*) *intermedium*. In order to accommodate the subgenus *Fibricola*, the generic diagnosis of *Neodiplostomum* by Dubois (1953) must be emended to read: vitellaria in both fore- and hindbody or confined to forebody, bisegmentation of body more or less well marked, parasitic in birds and mammals. The three subgenera can be separated as follows:

A. Genital cone present; anterior testis usually symmetric. Subgenus **Conodiplostomum**
AA. Genital cone absent; anterior testis usually asymmetric.
B. Vitellaria abundant in hindbody; parasitic in birds. Subgenus **Neodiplostomum**
BB. Vitellaria reduced or absent in hindbody; parasitic in mammals. Subgenus **Fibricola**

In connexion with the above proposals concerning *Neodiplostomum* and *Fibricola*, it is important that the type species be well known and the type material available. The type species of *Neodiplostomum* (*Conodiplostomum*) is *N. spathula* (Creplin), a well-known species, with type material available. The type species of *Neodiplostomum* and hence of *Neodiplostomum* (*Neodiplostomum*), designated by Railliet

(1919) as type of his new genus, *Neodiplostomum*, is *N. spathulaeforme* (Brandes). Brandes (1890) briefly described this species from a single immature specimen, which has since been lost. The species has never been found again and redescribed, probably because the original and only description is inadequate. To remedy this situation, Dubois (1953) proposed *N. spathoides* Dubois as the type species, and placed *N. spathulaeforme* in the category of *species delineatae*. As Bisseru (1956) has pointed out, this action of Dubois contravenes the International Rules and *'species delineatae'* have no recognition in the Rules. However, if *N. spathulaeforme* is recognized as a *nomen dubium* and subsequently placed on the list of rejected specific names, then it cannot act as type species but must be replaced by another species, preferably *N. spathoides*. That *N. spathulaeforme* should be considered a *nomen dubium* is evident since the existing description is such that no specimens could be assigned to the name and the type material is lost.

SUMMARY

Neodiplostomum intermedium n.sp. from the small intestine of the allied rat, *Rattus assimilis*, is described. It can be separated from other species of *Neodiplostomum* by the following combination of characters: genital cone absent; vitellaria form two ventro-lateral bands in hindbody without terminal masses; anterior testis nearly symmetric, as wide as posterior testis. It is proposed that *Fibricola* be made a subgenus of *Neodiplostomum* containing the species in *Fibricola* and the species, *N. intermedium* described herein, because *N. intermedium* is intermediate between *Neodiplostomum* (*Neodiplostomum*) and *Fibricola*. Further evidence is given in favour of the invalidation of the subfamily Alariinae.

This study was supported by a grant from the Rural Credits Fund of the Commonwealth Bank of Australia. Thanks are due to Professor J. F. A. Sprent, University Veterinary School, for his criticism of the manuscript, and to Mr K. Webber and his sons for their aid in trapping rats.

REFERENCES

Barker, F. D. (1915). Parasites of the American muskrat (*Fiber zibethicus*). *J. Parasit.* **1**, 184–97.

Bisseru, B. (1956). Three new species of the genus *Neodiplostomum* Railliet, 1919, from Central African birds of prey, with a note on *Neodiplostomum canaliculatum* (Nicoll, 1914) Dubois, 1937. *J. Helminth.* **30**, 51–62.

Brandes, G. (1890). Die Familie der Holostomiden. *Zool. Jahrb. Abt. Syst.* **5**, 549–604.

Chandler, A. C. (1942). The morphology and life cycle of a new strigeid, *Fibricola texensis*, parasitic in raccoons. *Trans. Amer. Micr. Soc.* **61**, 156–67.

Chandler, A. C. & Rausch, R. (1946). A study of strigeids from Michigan mammals with comments on the classification of mammalian strigeids. *Trans. Amer. Micr. Soc.* **65**, 328–37.

Dubois, G. (1932). Révision des 'Hémistomes' et étude de formes nouvelles. *Bull. Soc. neuchât. Sci. nat.* **56**, 375–412.

Dubois, G. (1936). Nouveaux principes de classification des Trématodes du groupe Strigeida. *Rev. suisse Zool.* **43**, 507–15.

Dubois, G. (1937). Études de quelques Strigéidés d'Australie et notes sur le genre *Fibricola* Dubois, 1932. *Ann. Parasit. hum. comp.* **15**, 231–47, 333–53.

DUBOIS, G. (1938). Monographie des Strigeida (Trematoda). *Mém. Soc. neuchât. Sci. nat.* **6**, 1–535.

DUBOIS, G. (1944). À propos de la spécificité parasitaire des Strigeida. *Bull. Soc. neuchât. Sci. nat.* **69**, 5–103.

DUBOIS, G. (1953). Systématique des Strigeida. *Mém. Soc. neuchât. Sci. nat.* **8**, 1–141.

DUBOIS, G. & RAUSCH, R. (1948). Seconde contribution à l'étude des 'Strigeides' ('Trematoda') nord-américains. *Bull. Soc. neuchât. Sci. nat.* **71**, 29–61.

DUBOIS, G. & RAUSCH, R. (1950). Troisième contribution à l'étude des Strigeides (*Trematoda*) nord-américains. *Bull. Soc. neuchât. Sci. nat.* **73**, 19–50.

LA RUE, G. R. & BOSMA, N. J. (1927). Studies on the trematode family Strigeidae (Holostomidae). *Neodiplostomum lucidum*n.sp. *J. Parasit.* **14**, 124–5.

MILLER, M. J. (1940). A new trematode, *Fibricola laruei*, from the raccoon in Canada. *Canad. J. Res.* (Sect. D.), **18**, 333–5.

PEARSON, J. C. (1956). Studies on the life cycles and morphology of the larval stages of *Alaria arisaemoides* Augustine & Uribe, 1927 and *Alaria canis* La Rue & Fallis, 1936 (Trematoda: Diplostomidae). *Canad. J. Zool.* **34**, 295–387.

RAILLIET, A. (1919). Nouveaux trématodes du chien. Abstract of Hall, M. C., and Wigdor, M. (1919 *g*). *Rec. Méd. Vét.* **95**, 229–32.

READ, C. P. (1948). Strigeids from Texas mink with notes on the genus *Fibricola* Dubois. *Trans. Amer. Micr. Soc.* **67**, 165–8.

ULMER, M. J. (1955). Notes on the morphology and host-parasite specificity of *Fibricola cratera* (Barker & Noll, 1915) Dubois, 1932 (Trematoda: Diplostomitadae). *J. Parasit.* **41**, 460–6.

(*MS. received for publication* 20. III. 1958.—Ed.)

THE IDENTITY OF THE LARVAL NEMATODES FOUND IN THE BODY MUSCLES OF THE COD (*GADUS CALLARIAS* L.)

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(With 15 Figures in the Text)

INTRODUCTION

There is a very extensive literature on the larval nematodes found in cod (*Gadus callarias* L.). As this has been recently summarized by Dollfus (1953), it is not proposed to review it here. There has been very considerable confusion in the past over the identification of these nematodes, the main difficulty being that few of the adult characters are to be found in the larvae. Some of the larvae which have been found have an oesophagus divided into two parts (an anterior muscular portion and a posterior glandular part) and, in addition, have caeca in the digestive tract. When these are present it is often possible to assign the larva to a genus. With *Contracaecum*, for instance, very young larvae show, like the adult, one caecum extending anteriorly from the front of the intestine and another extending posteriorly from the glandular part of the oesophagus (Dollfus, 1953). Young larvae do not always, however, have the same form of gut as the adult. In *Porrocaecum* larvae smaller than 28 mm. the intestinal caecum found in the adult has not yet developed (Baylis, 1916). Thus, larvae showing a division of the oesophagus into two parts, but having no gut caeca at all, may belong to any of several genera. Such larvae are unfortunately common in the body musculature of cod. They have been ascribed principally to three genera—*Anisakis*, *Eustoma* (=*Acanthocheilus*) and *Porrocaecum* (Dollfus, 1953).

The object of this study was to identify as far as possible the larval nematodes found in the epaxial and hypaxial muscles of cod. The larval nematodes which are found in the viscera are not considered here.

LARVAE FROM COD BODY MUSCLE

It was found by means of culture experiments that the larvae in cod muscle belong to two genera: *Porrocaecum* and *Anisakis*. Since there has been so much confusion over the identity of such larvae in the past, detailed descriptions are now given of each. All the forms described in this report were obtained from Icelandic cod. These fish had been stored on ice for a number of days. In spite of this the larvae became very active when they were removed from their cysts and kept at room temperature for a short time.

(*a*) *Description of larval* Porrocaecum

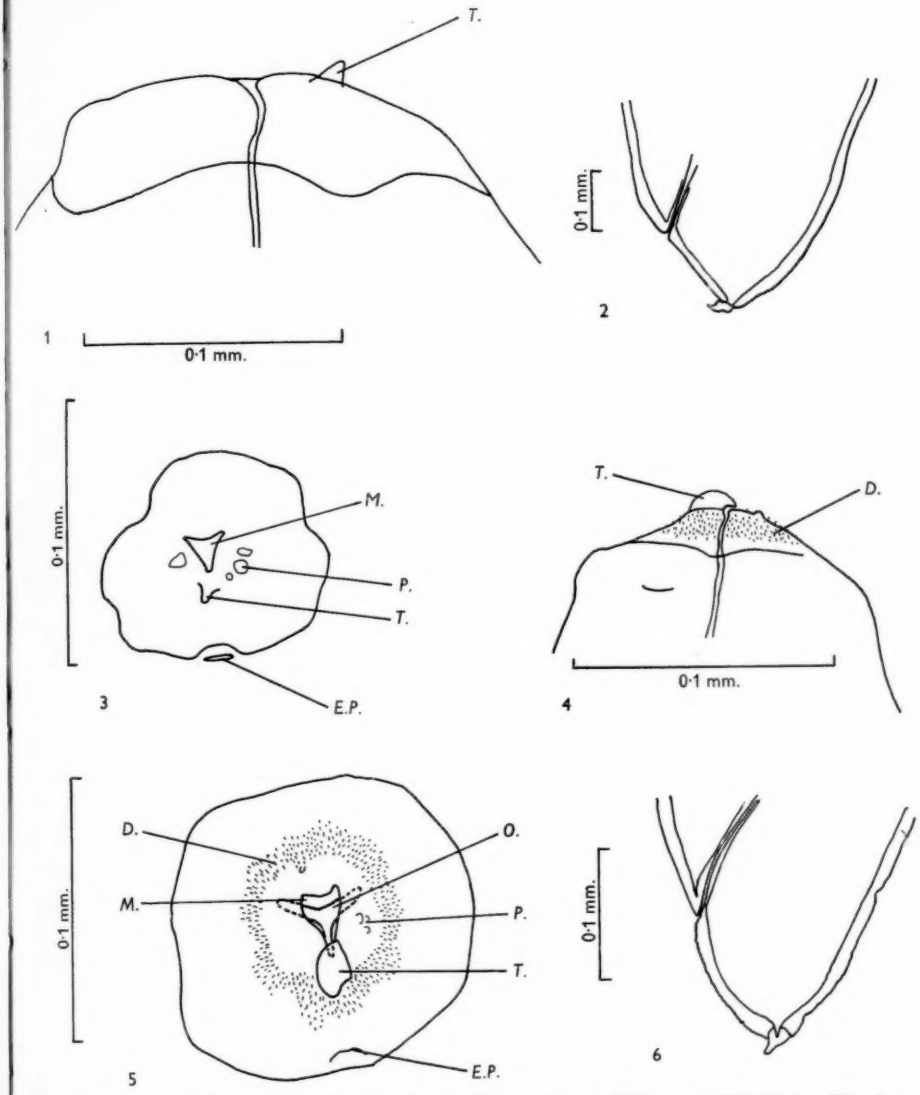
These larvae were found coiled, usually in a spiral inside a capsule, embedded in the musculature of the cod. The capsule is thin and membranous. According to Baylis (1916), the worm itself is usually surrounded by one or more cast-off cuticles distinct from the capsule wall. I can confirm this. In a few cases the front part of the worm was wound in one spiral, and the posterior part coiled into another. A few worms were found in the act of penetrating the musculature from the body cavity of the cod.

The living worm is usually whitish or reddish in colour. There is considerable variation in the extent of the red coloration, and it is frequently confined to the posterior part of the body. It may be due to haemoglobin, and it is principally found in the cells lying between the gut and the body-wall musculature.

There was some variation in the length of the worms: the majority of them were between 35 and 48 mm. in length. All the measurements given in this paper were made on worms which had been killed in hot 70% alcohol and cleared in lacto-phenol. Some of the worms were slightly more extended and some had slightly contracted by this treatment, so that the range of measurements in each case is probably rather greater than in the living worms. The maximum width varied between 0·7 and 1·0 mm. The body cuticle was smooth, except for fine, incomplete transverse striations. The presence of these seemed to depend on the tension on the cuticle. In places where the worm was bent and the cuticle was thus somewhat stretched, no striations were seen.

The mouth is surrounded by an undivided lip mass (Figs. 1, 3). It is, however, possible to distinguish the position of the three future lips by the indentations at the edge of the fused lip mass and also by reference to the ventral situation of the excretory pore which lies adjoining the fused lips (Fig. 3). The mouth is triangular and on the lip mass surrounding it are 3 or 4 blunt papillae. These papillae may perhaps be artifacts due to fixation. A conspicuous boring tooth lies on the lip mass on the mid-ventral side. This is in contradiction with Baylis (1916) who states that it is lateral in position. No fine teeth can be distinguished on the lip mass. At the extreme posterior end of the body is a small papilla-like process (Fig. 2).

The oesophagus is divided into two parts, an anterior muscular, and a posterior glandular part. The length of the muscular portion varied between 2·2 and 2·8 mm. The glandular portion is shorter, and varied between 1·0 and 1·4 mm. There is an oblique junction between the oesophagus and the intestine (Fig. 8). The length of the caecum lay between 0·6 and 1·2 mm. but in any individual worm it was slightly shorter than the glandular part of the oesophagus (= ventriculus). In the living worm the intestine and caecum and usually the ventriculus as well (but not the muscular part of the oesophagus) were opaque, and in transmitted light looked black. In preserved and cleared specimens, however, this opaqueness was lost, and the intestine became a very pale brown colour. In most cleared specimens it was not easy to distinguish the caecum. Consequently, the gut must be dissected out in order to be sure that the caecum is present. The importance of this cannot be over-emphasized. It can readily be done by cutting the worm into pieces and



Figs. 1–3. Larva of *Porrocaecum* from cod musculature. Fig. 1. Side view of lip mass. Fig. 2. Tail. Fig. 3. Front view of lip mass. *E.P.*, Excretory pore; *M*, mouth; *P.*, papillae; *T.*, boring tooth.

Figs. 4–6. Larval *Anisakis* from cod body muscle. Fig. 4. Side view of lip mass. Fig. 5. Front view of lip mass. Fig. 6. Tail. *D.*, Denticles; *E.P.* excretory pore; *M.*, mouth; *O.*, oesophageal opening; *P.*, papillae; *T.*, boring tooth.

squeezing out the gut with a fine needle. The tip of the caecum is attached to the body wall by a number of fibrous strands (Fig. 8).

I have compared the larval *Porrocaecum* described above, obtained from the musculature of Icelandic cod, with specimens (preserved in glycerine-alcohol) from cod caught off the east coast of Canada, and kindly sent by Dr Ronald. I am unable to find any difference between the larvae from these two sources.

(*b*) *Description of larval Anisakis*

These worms, like the *Porrocaecum*, were usually found coiled in a spiral, enclosed in a thin-walled cyst embedded in the musculature of the cod. When removed from the capsule they are in most cases completely colourless, except for the glandular part of the oesophagus, which is white and opaque. In transmitted light this appears black. Occasionally specimens are found which show a slight reddish coloration in the posterior part of the body. The cuticle is smooth, except for slight striations at intervals, but the presence of these seemed to depend to some extent on the state of extension of the worm. The larvae were on the whole smaller than those of *Porrocaecum*, having an average length (in preserved specimens) of between 21 and 33 mm. and a maximum width of between 0·49 and 0·55 mm.

The mouth is surrounded by a lip mass (Figs. 4, 5). The positions of the future lips cannot be discerned on this. On the lip mass are usually found a few scattered rather flat papillae. These, like those of *Porrocaecum*, may be artifacts due to fixation. The mouth opening is more or less triangular, the apex of the triangle pointing towards the ventral side. On the lip mass on the ventral side lies a very prominent boring tooth. The edge of the lip mass is covered with large numbers of minute teeth or hairs. There does not appear to be a sharp division between the lip mass and the rest of the body. The excretory pore lies just outside the border of fine teeth on the ventral side. At the extreme posterior end of the body is a small papilla (Fig. 6).

The oesophagus consists of two portions—an anterior muscular part and a posterior glandular part. The length of the former lay between 2·4 and 2·6 mm. The length of the latter lay between 0·7 and 0·8 mm. When cleared in lactophenol the glandular part becomes completely transparent like the muscular part. The oesophagus and intestine are straight in the living worm. On dissection it was found that no trace of a caecum (intestinal or oesophageal) was present.

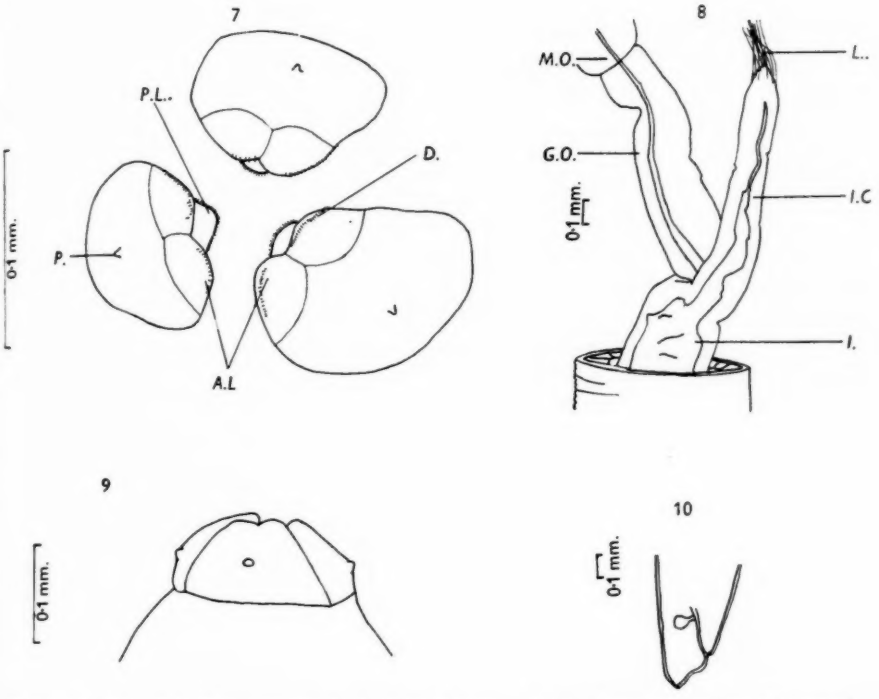
RESULTS OF CULTURE EXPERIMENTS

As the identification of the larval worms, particularly the smaller ones, is almost impossible when they are in the larval state, it was decided to try and get them to an identifiable state by culturing them in test tubes.

Martin (1921) succeeded in inducing two *Porrocaecum* larvae from the musculature of *Osmerus eperlanus* to moult to the pre-adult state in culture, by keeping them in the following solution at 38° C.: 100 g. of 0·2 % HCl solution + 30 drops of a pepsin-glycerine extract of rennet + ½ c.c. of pieces of raw fish. From his description it is probable that the pre-adults were abnormal. This may have

been due to the severe fungus infection which he mentioned was found in the cultures.

The presence of an intestinal caecum in some of the present larval worms suggested that they belonged to the genus *Porrocaecum*. Since there is some evidence that *Porrocaecum* larvae from the flesh of Canadian cod become adult in the stomach of the harbour seal (*Phoca vitulina*) (Scott, 1953), cultures were run at 37° C. and



Figs. 7–10. Pre-adult *Porrocaecum* obtained by culture. Fig. 7. Front view of lips. The mouth and excretory pore are not shown. Fig. 8. Part of the gut dissected out. Fig. 9. Side view of lips. Fig. 10. Tail. *A.L.*, Anterior lobes; *D.*, denticles; *G.o.*, glandular part of oesophagus; *I.*, intestine; *I.C.*, intestinal caecum; *L.*, ligaments; *M.O.*, muscular part of oesophagus; *P.*, papilla; *P.L.*, posterior lobe.

the buffer solution used had a low pH. These conditions were thus similar to those employed by Martin (1921). The buffer used throughout consisted of 0·4 ml. 0·2 M-Na_2HPO_4 and 19·6 ml. of 0·1 M citric acid. This had a pH of 2·2. Some experiments were carried out at pH 7·2 (17·4 ml. 0·2 M-Na_2HPO_4 + 2·6 ml. 0·1 M citric acid) in the presence of 0·1 % trypsin. The worms in these experiments died in 2 days, probably because of the heavy bacterial growth in the tubes. In all the experiments at pH 2·2 bacterial contamination was negligible. In a few cases there was some slight growth of a yeast in the tubes, but this had no apparent effect on the worms. In most cases the media were changed every 2 days. The tubes were kept in racks

immersed in a constant temperature bath, and were not shaken or artificially aerated. In the following account *Porrocaecum* and *Anisakis* larvae are treated together, as no difference was found between them in their culture behaviour.

(*a*) *Hatching from cysts*

When the cysts containing the larvae are removed from the cod muscle, the larvae lie quiescent inside. On being placed in the buffer solution at pH 2·2 at 37° C., the worms emerged within a few hours. Under the same conditions but with a commercial pepsin preparation added to a concentration of 1 % the worms all hatched within 10 min. Clearly the presence of the pepsin assists hatching, probably by softening the cyst.

On emergence from the cysts, the worms show great activity, and this persists for several hours. By the next day, however, they are much quieter.

(*b*) *Factors involved in the moult to the pre-adult state*

The effects of various conditions on the moult to the pre-adult state are given in Table 1. It is clear from this: (1) that the addition of pieces of fish to the tubes is essential for the moult to take place, (2) that, while pepsin assists hatching from the cysts, it is not essential for moulting, and (3) that a high temperature is essential for the moult to take place.

Table 1. *Effect of various culture conditions on moulting to the pre-adult stage*

Medium	Temp. (° C.)	No. larvae at start	No. moulted	Total dead by 5th day	Total dead by 10th day	No. living on 10th day
Buffer	37	8	0	3	5	3
Buffer + 1 % pepsin	37	28	0	10	22	6
Buffer + 0·5 % Lablemco	37	6	0	3	4	2
Buffer + 1 % pepsin + fish on day 1	37	9	7	0	2*	7*
Buffer + 1 % pepsin + fish on day 2	37	10	7	0	3*	8*
Buffer + 1 % pepsin + fish on day 4	37	9	2	1	6	3
Buffer + fish on day 1	37	9	4	0	2	7
Buffer + fish on day 1	16	9	0	0	2	7
Buffer + 1 % pepsin + fish on day 1	16	9	0	1	1	8

* Eighth day.

All the larvae used consisted of roughly equal numbers of *Porrocaecum* and *Anisakis* from Icelandic cod, with the exception of those noted in the last three lines of Table 1 which consisted of *Porrocaecum* only. These were obtained from cod fished off the west coast of Scotland (Butt of Lewis) and appeared to be identical with the Icelandic ones, both as larvae and as pre-adults.

Pieces of fish were added to the tubes only on the days mentioned. The fish was always removed after 24 hr., and the medium was then replaced by fresh solution.

Table 2 shows the effect of the time at which fish was given on the time at which

the moult occurred. It is clear, although the numbers are small, that the larvae do not moult until at least 3 or 4 days have elapsed from the time at which they were given food. All the experiments given in Table 2 were carried out at 37° C.

Table 2. *Influence of feeding on moulting to the pre-adult stage*

Days after beginning of experiment ...	1	2	3	4	5	6	7	8	9	10	11	Totals
No. worms moulting after being fed on day 1	Fed	0	0	2	3	2	0	0	0	0	0	7
No. worms moulting after being fed on day 2	—	Fed	0	0	0	5	1	0	1	0	0	7
No. worms moulting after being fed on day 4	—	—	—	Fed	0	0	0	1	0	1	0	2

DESCRIPTION OF PRE-ADULTS

(*a*) *Porrocaecum*

No marked change in length was observed when the worms moulted, nor was there any marked change in the main body cuticle. Irregular striations are found in this, but seem to depend on the degree of extension of the cuticle.

The most conspicuous change is in the differentiation of the lip mass into three distinct lips (Figs. 7 and 9). The anterior portion of each lip is markedly bilobed, and an almost continuous row of denticles runs along its edge. Behind these lobes is a single posterior lobe with a fine dentigerous margin. The ventro-lateral lips each bear a small papilla on their outer surface. The dorsal lip bears two papillae on its outer surface. These are often difficult to see. No interlabia are present.

At the extreme posterior end of the body the papilla, so conspicuous in the larva, has almost gone in the pre-adult (Fig. 10).

In the gut a marked change has taken place in the intestine, which now shows numerous irregular indentations and has lost the even cylindrical form found in the larva. No marked change is observable in the rest of the gut. The relative lengths of the two parts of the oesophagus and the caecum appear to be the same in the pre-adult as in the larva.

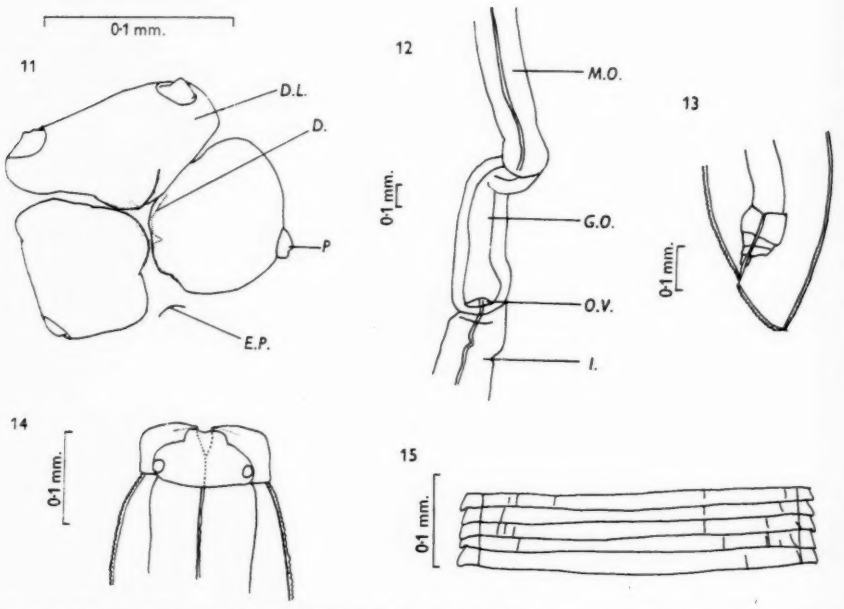
No cervical alae are present. If, however, the worm is dying, the cuticle frequently parts from the body wall, giving an appearance of alae. This is clearly what has happened in the worm figured by Martin (1921), in which conspicuous cervical alae were present.

I have closely examined the pre-adults, and I am unable to find the pre-anal or post-anal papillae which Martin (1921) claimed to have seen. In some individuals the developing vagina and uteri could be clearly seen. Attempts to rear the pre-adults to the fully adult state have so far failed.

(*b*) *Anisakis*

There is no marked increase in size following the moult from the larval to the pre-adult stage. The moulted animal shows a number of conspicuous differences

from the larva. The most obvious is the differentiation of the lips (Figs. 11 and 14). The dorsal lip is the largest and bears two large blunt papillae on its outer surface, and the two ventro-lateral lips each have a single large papilla on their outer surface. The inner part of each lip is rounded or has a slightly bilobed appearance and bears a line of fine denticles. No interlabia or posterior lip lobes are present.



Figs. 11–15 Pre-adult *Anisakis* obtained by culture. Fig. 11. Anterior view of lips. The mouth is not shown. Fig. 12. Part of gut dissected out. Fig. 13. Tail. Fig. 14. Side view of lips. Fig. 15. Surface view of body cuticle. *D.*, denticles; *D.L.*, dorsal lip; *E.P.*, excretory pore; *G.O.*, glandular part of oesophagus; *I.*, intestine; *M.O.*, muscular part of oesophagus; *O.V.*, oesophageal valve; *P.*, papilla.

The whole body cuticle bears regular distinct transverse lines giving the worm, when seen under high magnification, an almost segmented appearance (Fig. 15). The papilla at the extreme posterior end of the body is greatly reduced in size (Fig. 13).

A marked change is to be seen in the intestine which is no longer a smooth cylindrical tube, but now has many identations. The glandular part of the oesophagus in most specimens now has a distinct bend at its anterior end, so that the appearance of the posterior end of the oesophagus is almost sigmoid (Fig. 12). In a few preserved individuals, this part of the oesophagus is straight. No marked changes were observed in the relative proportions of the two parts of the oesophagus.

These pre-adults are very similar to those described by Dollfus (1948) as *Anisakis* sp. from the stomach of *Orca orca* (L.). Attempts to rear the pre-adults to the adult stage have, so far, failed.

DISCUSSION

The larger larvae taken from cod body muscle proved, when reared to the pre-adult state in culture, to belong to the genus *Porrocaecum* Raillet & Henry, 1912, subgenus *Terranova* Leiper & Atkinson, 1914, on the basis of the characters given above. Thirteen species of this sub-genus are known (Mosgovoy, 1951). On the basis of the morphological characters of the pre-adults it is not possible to assign them to any one of these species. The culture experiments (Table 1), however, seem to indicate that the present larvae become adult in a warm-blooded host; if this is so, the number of known species to which they might belong is immediately reduced to two (the other species all being found as adults in the gut of fish and reptiles), namely, *T. decipiens* (Krabbe, 1878) and *T. antarctica* (Leiper & Atkinson, 1914). Of these the second is known only from antarctic seals, but the first has been recorded from many seals on numerous occasions in the North Atlantic. It would seem, therefore, that this is the most likely species for the present larvae to belong to. This is supported by the work of Scott (1953), who claimed as a result of feeding experiments that certain nematode larvae found in the flesh of Atlantic cod became adult *Porrocaecum decipiens* (Krabbe, 1878) in the stomach of the harbour seal (*Phoca vitulina*).

The smaller larvae taken from the cod body muscle proved to belong to the genus *Anisakis* Dujardin, 1845, on the basis of the characters of the pre-adults, which are given above. Eighteen species of this genus are known (Mosgovoy, 1951), but it is not possible on the basis of the above characters to decide to which species the present specimens belong. The culture experiments suggest that the larvae become adult in a warm-blooded host. All the known species of *Anisakis*, however, have been recorded from mammals (whales, dolphins, seals). Lyster (1940) points out that this genus needs revision. Many of the species are poorly differentiated and specific determination of adults is often impossible. He unites three species with *A. simplex* (Rudolphi, 1809). If this is justified, it may well be that the present larvae belong to this species complex, since *A. simplex* (Rudolphi, 1809) has been recorded on numerous occasions from a wide variety of marine mammals in the North Atlantic and Arctic (Dollfus, 1953).

Most of the present work was carried out on worms taken from Icelandic fish. In these *Porrocaecum* and *Anisakis* were present in roughly equal numbers. In a sample of cod from the Butt of Lewis, Scotland, only *Porrocaecum* were found. These appeared to be identical with the Icelandic ones, both as larvae and pre-adults. No other nematodes were found encysted in the body muscles of cod from either locality.

Larvae were also collected from the body muscles of cod caught off Greenland and from some caught in the Barents Sea. No attempt was made to rear these to the pre-adult state. In both cases the samples consisted only of larvae which appeared identical with *Porrocaecum* and *Anisakis* from Icelandic fish. The latter were present in a great preponderance. It thus seems—although more culture experiments are necessary in order to prove this—that the nematode larvae taken from cod body muscle from these widely separated localities belong only to the genera *Porrocaecum* and *Anisakis*.

Some remarks on the significance of the moult to the pre-adult state are pertinent at this point.

When the larvae of *Porrocaecum* and *Anisakis* are ingested together with cod flesh by another animal, they presumably emerge from their cysts when they reach the stomach. If conditions are suitable, they moult within about 5 days to the pre-adult state (see Scott's (1953) experiments with the seal *Phoca vitulina*). They are now equipped with three differentiated lips, which would enable them to attach themselves to the gut wall and to grow to the adult state. If conditions are not suitable, they would either die or, retaining their larval characters (including the boring tooth), penetrate through the gut wall and re-encyst in the body muscles. Scott's (1954) work seems to show that this is what happens when cod eat Smelt (*Osmerus mordax*) flesh containing encysted *Porrocaecum*. His work suggests that the larvae hatch from their cysts when they reach the stomach, penetrate the gut wall and re-encyst in the main body muscles of the cod. One of the main factors which determine whether or not they moult is temperature (Table 1). Only when this is sufficiently high (as in the case inside marine mammals) will they moult to the next stage in the life cycle. In cold-blooded hosts one would expect them to be passed from one host to another, encysting each time, until they ultimately die.

The question is now raised as to whether the larvae are viable in warm-blooded animals apart from marine mammals. Four larvae (*Anisakis* + *Porrocaecum*) were force-fed to each of six young Wistar Albino rats, six young kittens and six pigeons. Fourteen days later these were killed and the whole gut examined for nematodes. None belonging to the present genera was found. This suggests that the worms (none of which was destroyed by the rat chewing them) are not viable in all mammals.

Neither *Porrocaecum* nor *Anisakis* adults have yet been recorded from man. (Hitchcock (1950), who examined the faeces of 100 Eskimos in Alaska, found that 10 % of them showed Ascarid larvae. Of the specimens collected, one 'was definitely identifiable to the subfamily Anisakinae, probably to the genus *Porrocaecum*; and another could, with reasonable certainty be determined as a member of the genus *Anisakis*'.

The work described in this paper was carried out as part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research, at the Humber Laboratory in Hull.

SUMMARY

Larval nematodes occurring in cysts in the body musculature of Icelandic cod have been identified as belonging to *Porrocaecum* Raillet & Henry, 1912 (subgenus *Terranova* Leiper & Atkinson, 1914), probably *P. decipiens* Krabbe and *Anisakis* Dujardin, 1845. Identification was made possible by rearing them *in vitro* to the pre-adult condition. The experiments show that the following conditions are necessary for the moult to the pre-adult state; (1) a fairly high culture temperature (37° C.); (2) the presence of pieces of fish for 24 hr. towards the beginning of the culture period. 1 % pepsin, while it assists the hatching from the cysts, is

not essential for the moult to the pre-adult condition. Experiments indicate that the moult takes place 3–5 days after fish has been supplied.

The larvae and pre-adults of both genera are described. Samples of larvae from cod from Greenland and the Barents Sea probably consist only of *Porrocaecum* and *Anisakis*, but a sample from West Scotland contained only *Porrocaecum*. The significance of the moult to the pre-adult state is discussed.

REFERENCES

Baylis, H. A. (1916). Some Ascarids in the British Museum (Natural History). *Parasitology*, **18**, 360–78.

Baylis, H. A. (1944). 'Capsularia Marina' and the Ascarids of Marine hosts. *Parasitology*, **36**, 119–21.

Dollfus, R. Ph. (1948). Nématode à oesophage sigmoide de l'éstomac d'une *Orca orca* (L. 1789) (Cétace odontocète). Liste des *Anisakis* des cétacés et des pinnipèdes. *Ann. Parasit. hum. comp.* **23**, 305–22.

Dollfus, R. Ph. (1953). *Aperçu général sur l'histoire naturelle des parasites animaux de la morue atlanto–arctique* Gadus callarias L. (= *morrhua* L.). Paris, Lechevalier. 423 pp.

Hitchcock, D. J. (1950). Parasitological study on the Eskimos in the Bethel area of Alaska. *J. Parasit.* **36**, 232–4.

Lyster, L. L. (1940). Parasites of some Canadian sea mammals. *Canad. J. Res.* **18**D, 395–409.

Martin, O. (1921). Über Ascaridenlarven aus dem Fleische von Seefischen. *Z. Infekt. Kr. Haustiere*, **22**, 13–36.

Mosgovoy, A. A. (1951). In Skrjabin, K. I., Shikhobalova, N. P. & Mosgovoy, A. A., *Descriptive Catalogue of Parasitic Nematodes*, vol. 2. *Oxyurata and Ascaridata*. Moscow. 632 pp. (In Russian.)

Scott, D. M. (1953). Experiments with the harbour seal, *Phoca vitulina*, a definitive host of a marine nematode, *Porrocaecum decipiens*. *J. Fish Res. Bd. Can.* **10**, 539–47.

Scott, D. M. (1954). Experimental infection of Atlantic Cod with a larval marine nematode from Smelt. *J. Fish. Res. Bd Can.* **11**, 894–900.

(*MS. received for publication* 28. III. 1958.—Ed.)

THE INCIDENCE AND DISTRIBUTION IN BRITAIN OF THE TREMATODES OF *TALPA EUROPAEA*

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INTRODUCTION

The trematodes harboured by the mole, *Talpa europaea*, have received but scant attention. There are three intestinal species, all of which occur in Britain, but the records of their distribution are very limited. Thus Davies (1932) and James (1954) have reported *Itygonimus lorum* and *I. ocreatus* from west Wales and Fahmy (unpublished thesis) recorded the same two species from south-east Scotland. In addition, Davies (1932) mentioned the third species, *Omphalometra flexuosa*, as occurring in west Wales. The present work, though by no means exhaustive, fills in the picture to some extent by providing records from moles caught in four main areas: Suffolk, Cheshire–Staffordshire, Westmorland–Yorkshire, south-west Scotland, and a single record from Oxford.

Although they happen to provide a fair selection of soil types and farming conditions, these areas were not chosen deliberately, but either because the author had the opportunity of trapping there or through the willing co-operation of local mole catchers and scientific workers.

It is, therefore, my pleasant duty to acknowledge with gratitude the assistance rendered by Air-Commodore the Hon. J. D. Boyle, C.B.E., the Earl of Cranbrook, C.B.E., Dr A. N. Frankland, D.F.C., and also by the mole catchers, Messrs Bailey, Clark, Faulds and Pratt. Particular thanks are due to Dr G. Crowcroft who provided nearly all the Suffolk material.

MATERIAL AND METHODS

Whenever possible moles were examined in a fresh condition. The length and sex of each mole were recorded and a note made of the locality and type of ground from which it came. The gut was then removed and the stomach separated from the intestine. Since the intestine presents no gross morphological differentiation, it was simply cut in half to give upper and lower intestines. These three sections of the gut were then opened and decanted in water separately, the washed residue of each being examined in a Petri dish under low-power binoculars. A note was made of any identifiable food particles and of all helminths found.

Moles which had to be preserved before examination were stored in formalin, the abdominal cavity having been opened to allow penetration of the fixative. The gut was later treated in the same way as fresh material. Much of the preserved material from Suffolk consisted only of intestines, the stomachs having been used for other purposes, but this does not affect the trematode and cestode record as these groups only occur in the intestine of the mole.

RESULTS

Species found

Three species of trematode were discovered in the moles examined. They were *Itygonimus lorum* (Dujardin, 1845), *I. ocreatus* (Goeze, 1782), and *Omphalometra flexuosa* (Rudolphi, 1809).

Sex of host

The sex ratio of the catch among the 593 moles whose sex was determined and of those among them parasitized with flukes is shown in Table 1.

There is, therefore, no difference between the sexes in the rate of infestation with flukes.

Table 1. *Sex ratio of total catch and parasitized moles*

	Moles examined		Moles with flukes	
Sex	No.	Ratio	No.	Ratio
♂	278	1	33	1
♀	315	1·13	38	1·15

Site of parasitism in the mole

The site of parasitism for the three species is shown in Table 2.

Table 2. *Site of parasitism of flukes in moles*

	Site of parasitism			
Species	Upper intestine	Lower intestine	Upper and lower intestine	Notes on cases from upper and lower intestine
I. lorum	0	86	1	1 fluke in upper intestine and 9 in lower intestine
I. ocreatus	77	4	8	4 moles fairly heavily infested
O. flexuosa	44	1	6	3 moles very heavily infested

I. lorum, therefore, is almost exclusively a parasite of the lower intestine, whereas *I. ocreatus* and *O. flexuosa* are predominantly parasites of the upper intestine.

Degree of infestation per mole

The degree of infestation per mole is shown for each species of fluke in Table 3.

Table 3. *Degree of infestation with flukes per mole*

Species	No. moles	Range	Mean	Mode
I. lorum	85*	1–29	4·7	1 (2 nearly as common)
I. ocreatus	78*	1–many	—	1 (predominantly)
O. flexuosa	48*	1–129	7·4	1 (predominantly)

* A few parasitized moles omitted as the number of flukes present could not be ascertained.

Super-imposed infestations

When immature as well as adult flukes of a species were found in one mole, it was considered reasonable to suppose that successive infestations had occurred.

Among eighty-seven moles infested with *I. lorum*, eleven contained immature as well as adult flukes. The presence together of immature and adult *I. ocreatus* was, however, only observed in three out of ninety infested moles, and no immature *O. flexuosa* were observed among fifty-one moles harbouring this species. It must be noted that the majority of the moles infested with *I. ocreatus* and *O. flexuosa* were examined in a preserved condition and this might have led to very small flukes being concealed in coagulated mucus.

Size differences between adults found in the same mole were observed in all three species and these, again, may be suggestive of successive infestations.

Incidence and geographical distribution

A study of the overall incidence of the three species of fluke shows *I. ocreatus* to be the most commonly found with *I. lorum* a close second, and *O. flexuosa* considerably less common. The figures are given in Table 4.

Table 4. *Overall incidence of mole flukes*

	I. lorum		*I. ocreatus*		*O. flexuosa*	
Moles examined	No.*	%†	No.*	%†	No.*	%†
1734	87	5·0	90	5·2	51	2·9

* Flukes present.
† Percentage of moles infested with flukes.

However, when the catch is divided up on a geographical basis, it is seen that the relative rates of incidence of the three species vary sharply as shown in Table 5.

Table 5. *Area distribution of mole flukes*

		I. lorum		*I. ocreatus*		*O. flexuosa*	
Area	Moles examined	No.*	%†	No.*	%†	No.*	%†
Suffolk	884	12	1·4	90	10·2	48	5·4
Cheshire–Staffs.	445	8	1·8	0	0	3	0·7
Westmorland–Yorks.	207	49	23·7	0	0	0	0
S.W. Scotland	97	17	17·5	0	0	0	0
Oxford	1	1	(100·0)	0	0	0	0

* Flukes present.
† Percentage of moles infested with flukes.
0 = No flukes found.

From Table 5 it is seen that *I. lorum* has a very high incidence in the Westmorland–Yorkshire area and only rather less so in south-west Scotland. In the Cheshire–Staffordshire and Suffolk areas its incidence is very low. The position of *I. lorum* is different from that of the other two flukes in that it was found in all the areas sampled, whereas *I. ocreatus* and *O. flexuosa* have a much more limited distribution as also shown in Table 5.

On a more detailed analysis the distribution, even within each main area, is extremely local. This is shown in Table 6*a–d* where, unless a locality has a name of its own, it is designated by a nearby village or river flowing through it. Land type for each main area is briefly indicated.

Table 6*a*. *Local distribution of mole flukes*

(Suffolk. Mainly fen peat.)

Area	No. moles examined	*I. lorum*		*I. ocreatus*		*O. flexuosa*	
		No.*	%†	No.*	%†	No.*	%†
R. Lark	228	0	0	3	1·3	7	3·1
R. Little Ouse and New Fen	223	0	0	9	4·0	16	7·2
Brandon Creek	23	0	0	0	0	0	0
R. Wissey	370	10	2·7	78	21·0	22	6·0
Saxmundham	6	1	16·7	0	0	0	0

Table 6*b*. *Local distribution of mole flukes*

(Cheshire–Staffordshire. Mainly lightish land.)

Area	No. moles examined	Moles with flukes		
		Species	No.*	%†
Keele	29	*I. lorum*	4	13·8
Kinderton	17	*I. lorum*	3	17·7
Other areas	252	—	0	0

Table 6*c*. *Local distribution of mole flukes*

(Westmorland–Yorkshire. Peaty and sour except King's Meaburn.)

Area	No. moles examined	*I. lorum*	
		No.*	%†
Fell End	21	1	4·8
Mallerstang	2	0	0
Garsdale	176	46	26·1
King's Meaburn	7	2	28·6

Table 6*d*. *Local distribution of mole flukes*

(South-west Scotland. Medium-light land and sour upland grazing.)

Area	No. moles examined	*I. lorum*	
		No.*	%†
Baldernock	15	1	6·7
Saltcoats	22	0	0
Dalry	62	14	22·6
Stewarton	38	0	0
Kilbirnie	39	0	0
Auchenmade	16	1	16·7
Portpatrick	2	0	0
[Oxford (clay)	1	1	100·0]

* Flukes present.

† Percentage of moles infested with flukes.

0 = No flukes found.

Seasonal occurrence

It was not possible to obtain a regular number of moles from each area throughout the year and this factor must be taken into account when considering variations in the seasonal occurrence of the flukes. It is therefore necessary to consider the monthly figures on a regional, as well as an overall, basis and these are shown for the three species respectively in Tables 7–9.

From these tables it is seen that, although there are sharp monthly fluctuations in the rate of infestation, the overall and regional variations do not correspond. Reference to Table 6*a–d* will show that, even within the regions, the figures largely depend on the local areas from which the monthly catch is made up.

Mixed infestations

Instances of simultaneous infestation of a mole with more than one species of fluke were rare. Thus among 226 moles carrying flukes only two were harbouring more than one species of fluke. Of these one was infested with *I. lorum* and *O. flexuosa* and the other with *I. ocreatus* and *O. flexuosa*. No case was observed of *I. lorum* and *I. ocreatus* being present together in the same mole.

It must be remembered, however, that many of the moles were collected in regions in which only one or two species of fluke occurred, so that the opportunities for multiple infestation were reduced. Of the 226 infested moles, 148 came from Suffolk, where all three species of fluke were known to occur, and a further eleven from Cheshire–Staffordshire where two species of fluke occurred. Of the 148 from Suffolk, 108 came from local regions, where the three species had been found and another forty-three from local regions, where two species had been recorded. Only in one local region, River Wissey, was there a possibility of *I. lorum* and *I. ocreatus* occurring in one mole. However, 108 moles from this area were found to contain either *I. lorum* or *I. ocreatus* and yet none contained both *Itygonimus* species.

Relationship between fluke infestations and other intestinal helminths

Some of the moles examined were parasitized by intestinal cestodes or nematodes. In Table 10 a comparison has been made between moles with and without trematodes of the relative rates of infestation with cestodes and nematodes.

It is seen from this table that the presence or absence of flukes in moles has no significant effect on the rate of infestation with other helminths.

DISCUSSION

Species found

The species found during the present work are the same three that have been recorded in Britain by earlier workers (Davies, 1932; James, 1954; Fahmy, unpublished thesis).

Sex of host

It is not to be expected that the sex of the host would have any effect on parasitism by intestinal trematodes, and this is borne out by the present findings.

Table 7. *Seasonal occurrence of* Itygonimus lorum

Month	Overall			Suffolk			Cheshire–Staffs.			Westmorland–Yorks.			S.W. Scotland		
	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†
January	80	6	7·5	56	0	0	16	2	12·5	8	4	50·0	—	—	—
February	103	7	6·8	55	0	0	30	1	3·3	18	6	33·3	—	—	—
March	125	11	8·8	70	0	0	21	0	0	27	9	33·3	6	1	16·7
April	118	13	11·0	48	1	2·1	30	0	0	32	12	37·5	8	0	0
May	480	26	5·4	199	0	0	107	3	2·8	30	7	23·3	144	16	11·1
June	218	3	1·4	126	0	0	37	0	0	16	3	18·8	39	0	0
July	53	3	5·7	47	2	4·3	1	1	100·0	3	0	0	—	—	—
August	86	6	7·0	81	5	6·2	2	0	0	3	0	0	—	—	—
September	131	1	0·8	19	1	5·3	84	0	0	28	0	0	—	—	—
October	163	2	1·2	102	0	0	36	1	2·8	27	1	3·7	—	—	—
November	104	5	4·8	50	0	0	46	0	0	8	5	62·5	—	—	—
December	64	3	4·7	34	1	2·9	23	0	0	7	2	28·6	—	—	—

* Flukes present.
† Percentage of moles infested with flukes.
0 = No flukes found.
— = No records made.

Table 8. *Seasonal occurrence of Itygonimus ocreatus*

	Overall			Suffolk			Cheshire–Staffs.			Westmorland–Yorks.			S.W. Scotland		
Month	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†
January	80	12	15·0	56	12	21·4	16	0	0	8	0	0	—	—	—
February	103	1	1·0	55	1	1·8	30	0	0	18	0	0	—	—	—
March	125	19	15·2	70	19	27·1	21	0	0	27	0	0	6	0	0
April	118	16	13·6	48	16	33·3	30	0	0	32	0	0	8	0	0
May	480	4	0·8	199	4	2·0	107	0	0	30	0	0	144	0	0
June	218	2	0·9	126	2	1·6	37	0	0	16	0	0	39	0	0
July	53	6	11·3	47	6	12·8	1	0	0	3	0	0	—	—	—
August	86	9	10·5	81	9	11·1	2	0	0	3	0	0	—	—	—
September	131	7	5·3	19	7	36·8	84	0	0	28	0	0	—	—	—
October	163	8	4·9	102	8	7·8	36	0	0	27	0	0	—	—	—
November	104	3	2·9	50	3	6·0	46	0	0	8	0	0	—	—	—
December	64	3	4·7	34	3	8·8	23	0	0	7	0	0	—	—	—

* Flukes present.
† Percentage of mole infested with flukes.
0 = No flukes found.
— = No records made.

Table 9. *Seasonal occurrence of Omphalometra flexuosa*

	Overall			Suffolk			Cheshire–Staffs.			Westmorland–Yorks.			S.W. Scotland		
Month	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†	No. moles examined	No.*	%†
January	80	0	0	56	0	0	16	0	0	8	0	0	—	—	—
February	103	10	9·7	55	10	18·2	30	0	0	18	0	0	—	—	—
March	125	2	1·6	70	2	2·9	21	0	0	27	0	0	6	0	0
April	118	1	0·8	48	1	2·1	30	0	0	32	0	0	8	0	0
May	480	5	1·0	199	5	2·5	107	0	0	30	0	0	144	0	0
June	218	6	2·8	126	3	2·4	37	3	8·1	16	0	0	39	0	0
July	53	6	11·3	47	6	12·8	1	0	0	3	0	0	—	—	—
August	86	13	15·1	81	13	16·0	2	0	0	3	0	0	—	—	
September	131	1	0·8	19	0	0	84	0	0	28	0	0	—	—	—
October	163	4	2·5	102	4	3·9	36	0	0	27	0	0	—	—	—
November	104	2	1·9	50	2	4·0	46	0	0	8	0	0	—	—	—
December	64	2	3·1	34	2	5·9	23	0	0	7	0	0	—	—	—

* Flukes present.
† Percentage of moles infested with flukes.
0 = No flukes found.
— = No record made.

Site of parasitism in the mole

Since the division of the intestine into upper and lower regions was by rough measurement only, it is evident from the fact that only one *I. lorum* was found in the upper intestine that this fluke normally occupies a position well into the lower half of the intestine. *I. ocreatus* is less rigidly confined to its commonest habitat, the upper intestine, and this probably means that it normally lives in the upper intestine but extends towards the central region. Davies (1932) made no mention of the location of these two species.

In the cases mentioned above the moles were not unusually heavily infested, but, of the six moles harbouring *O. flexuosa* in both upper and lower intestines, three had an exceptionally heavy worm burden. This suggests that although the upper intestine is the normal site for *O. flexuosa*, a finding confirmed by Davies's (1932) collection of his specimens from the 'small intestine', this worm is capable of living in the lower intestine and may be encouraged to do so by overcrowding in the upper intestine.

Table 10. *Trematode infestations in relation to cestode and nematode infestations*

(T = trematodes; C = cestodes; N = nematodes.)

Moles ± T	Total	−C or N	+C	+N	+C and N
Moles − T	1505	906	71	506	22
Moles + T	226	147	8	65	6
% moles − T	100	60	5	34	1
% moles + T	100	65	3	29	3

Degree of infestation per mole

The figures given in Table 3 indicate that although a host is capable of supporting a very large, or in the case of *I. lorum* moderate population of flukes, it is very much more usual for only one or two flukes to be present. Indeed, Davies (1932) did not come across heavy infestations in any of his moles. This may indicate a stable and well-balanced relationship between host and parasite, but it may, on the other hand, mean that moles do not feed heavily on the intermediate hosts and that these are usually only lightly parasitized.

Superimposed infestations

Evidence from the present findings suggests that premunition does not occur so far as *I. lorum* is concerned. Less evidence is available concerning *I. ocreatus*, but probably there is no premunition. The same may apply to *O. flexuosa*, although in this case the suggestion is based only on the presence of flukes of different sizes in the same mole, which could be due to differential rates of growth.

Incidence and geographical distribution

A study of the figures for incidence of mole flukes (Tables 4–6*a–d*) shows how misleading data may be if they are taken from one area. The highest figures are of the same order as those given by Davies (1932) for Cardigan, but the distribu-

tion is very local indeed and may vary enormously even between one field and another. The cause of this situation must lie in difficulties of transmission from one mole to another. Godfrey (1955) has reported moles remaining confined to quite a small area for 2–3 months at least, and, by their very nature, it would not be expected that they would range widely during their whole lives. If the intermediate hosts of mole flukes similarly have restricted movement, it might be expected that fluke infestations would be localized and patchy.

It is also probable that the requirements of the intermediate hosts restrict the distribution of mole flukes to soil types with certain characteristics. Gonder (1910) held the view that the restriction of *I. ocreatus* [called by him *I. lorum*, an error corrected by Davies (1932)] to moles from wet ground was due to the requirements of the intermediate host. Davies (1932) likewise found *I. ocreatus* at least more prevalent in moles from wet ground.

Information as to soil type is not sufficiently precise in the present work to draw any definite conclusion on the subject, but it does seem that the lightish land characteristic of most of the Cheshire–Staffordshire collection area is unsuited to some part of mole fluke life cycles. It is probably significant that Keele and Kinderton, the two local areas where flukes were found, have much heavier soil. The sour, peaty ground found in the Westmorland–Yorkshire collection area, and in the upland grazing area which produced most of the flukes from south-west Scotland, seems favourable to *I. lorum* but not to *I. ocreatus*, which nevertheless flourishes on the less acid peats along the river banks of the fens. The unifying factor in these areas may well be extra moisture, and the absence of *I. ocreatus* from the Westmorland–Yorkshire and south-west Scotland collecting areas may, speculatively, be accounted for by over-riding acidity.

Seasonal occurrence

The patchy geographical distribution discussed in the previous paragraph of necessity complicates the study of seasonal occurrence. The lack of correspondence in the overall and regional seasonal fluctuations shown in Tables 7–9 imply that there is no real seasonal variation in incidence of mole flukes but that these effects must be attributed to accidents of catch location. There is nothing in the present work to confirm Davies's (1932) tentative suggestion that *I. lorum* might be slightly more prevalent in February and March.

Mixed infestations

The evidence presented is sufficient to show that simultaneous infestation of a mole with more than one species of fluke is rare. It is possible for *O. flexuosa* to exist in company with *I. lorum* or *I. ocreatus*, but the data indicate that *I. lorum* and *I. ocreatus* are incompatible.

This is all the more remarkable when it is remembered: (1) that there seems to be no premunition in either species of *Itygonimus*, so that co-generic immunity cannot account for the situation and (2) that *I. ocreatus* and *O. flexuosa* are occupants of the same part of the intestine, whereas *I. ocreatus* and *I. lorum* are not.

Relationship between fluke infestation and other intestinal helminths

The fact that the presence of flukes in the mole intestine has no effect on the incidence of other helminths in the same site implies that flukes are not competing to any serious extent with other helminths for food or attachment, nor do they weaken the host in such a way that it is more vulnerable to attack by other helminths.

SUMMARY OF CONCLUSIONS

1. Moles were collected from four main areas, Suffolk, Cheshire–Staffordshire, Westmorland–Yorkshire, and south-west Scotland, and examined for the presence of flukes. Three species were found: *Itygonimus lorum* from all the areas (and in one mole from Oxford); *I. ocreatus* from Suffolk; *Omphalometra flexuosa* from Suffolk and Cheshire–Staffordshire.

2. The sex of the host has no effect on the incidence of parasitism with flukes.

3. *I. lorum* is almost exclusively a parasite of the lower half of the intestine, whereas *I. ocreatus* and *O. flexuosa* are predominantly parasites of the upper half of the intestine.

4. The commonest number of flukes of any of the three species to find in a mole is one, though two individuals is almost as common a number in the case of *I. lorum*. Nevertheless, large populations of *I. ocreatus* or *O. flexuosa* and moderate numbers of *I. lorum* are on occasion found in one mole.

5. Premunition does not occur with infestations of *I. lorum* and probably not with *I. ocreatus* and *O. flexuosa*.

6. The distribution of each species even within an area where it occurs is very patchy and local. This is probably bound up with restricted movement of the hosts, whether final or intermediate, and may also be associated with the influence of soil conditions on the intermediate hosts.

7. There appears to be no seasonal variation in the presence of flukes in moles.

8. Simultaneous infestation with more than one species of fluke is rare. It is possible for *O. flexuosa* to live in company with *I. lorum* or *I. ocreatus*, but the two *Itygonimus* species were never found together in the same mole.

9. The presence of flukes in a mole has no effect on the rest of the intestinal helminth population of the host.

REFERENCES

DAVIES, E. (1932). On a trematode, *Itygonimus lorum* (Duj. 1845), with notes on the occurrence of other trematode parasites of *Talpa europaea* in the Aberystwyth area. *Parasitology*, **24**, 253–8.

FAHMY, M. A. M. (1953). A study of intestinal parasites in small mammals. Unpublished Ph.D. Thesis presented to Edinburgh University.

GODFREY, G. K. (1955). A field study of the activity of the mole (*Talpa europaea*). *Ecology*, **36**, 678–85.

GONDER, R. (1910). *Itygonimus lorum* (Dujardin). *Zbl. Bakt.* (1. Abt.), **53**, 169–74.

JAMES, P. M. (1954). On some helminths from British small mammals, with a re-description of *Echinorhynchus rosai* Porta, 1910. *J. Helminth.* **28**, 183–188.

(*MS. received for publication* 29. III. 1958.—Ed.)

IMMUNITY IN TRYPANOSOMIASIS

III. SENSITIVITY OF ANTIBODY-RESISTANT STRAINS TO CHEMOTHERAPEUTIC DRUGS

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Whilst studying the development of neutralizing antibodies in animals infected with *Trypanosoma brucei*, Soltys (1957*a*) observed that only strains of *T. brucei* which were passaged frequently through mice and not exposed to antibodies could be inactivated by antibodies *in vitro* and become non-infectious to mice. Strains of *T. brucei* which were passaged through rabbits, and exposed to antibodies for some time, became antibody resistant, so that neutralizing antibodies had no effect upon them. It was also noticed that antibody-resistant strains differed in their sensitivity to trypanostatic drugs, although they had never before been exposed to any of the drugs. These latter observations suggested further studies on the relationship between antibody-resistant strains and drug-resistant strains. The results of these studies are presented in this paper.

MATERIALS AND METHODS

Strains. Trypanosoma brucei shinyanga III was used in these experiments. The strain has been maintained in mice and in rabbits by syringe passage. The strain which was passaged every second day through mice will be referred to as 'M' variant, whilst the strain which was passaged through rabbits every 25–28 days after infection will be referred to as 'R' variant. During a period of 25–28 days of infection the rabbits produced antibodies and the strain exposed to these antibodies became antibody resistant. Its antibody resistance was tested by the neutralization reaction described by Soltys (1957*a*). The sera of all rabbits through which the 'R' variant was passaged were examined for the presence of neutralizing antibody in order to be sure that the 'R' variant was exposed to antibodies.

The strains used for the tests were preserved by freezing to maintain them in an unchanged condition (Polge & Soltys, 1957), so that the same strain could be used throughout the same series of experiments.

The 'M' variant was made suramin fast by frequent passage through mice treated with subcurative doses of suramin. The subcurative dose was gradually increased until after seventeen passages (approximately 35 days) the strain was able to resist doses of 10 mg. of suramin. No attempt was made to increase the resistance further as 15–20 mg. doses were toxic to mice. *T. brucei* 'M' variant which was made suramin fast but not antibody fast will be referred to in the paper as 'D.F.' variant. The curative dose for mice infected with the normal 'M' variant was 0·1 mg. of suramin/20 g. body weight, but 10 mg. failed to cure mice infected

with 'D.F.' variant. This suramin-fast strain 'D.F.' was tested for its sensitivity to antibodies and to antrycide sulphate.

Drugs. Suramin, which is known under various synonyms (Bayer 205, Antrypol, Germanin, etc.), was used in all experiments, while antrycide sulphate was used only for therapeutic and trypanostatic effect *in vitro*, and antrycide prosalt was used for prophylactic experiments. The drugs were kindly supplied by I.C.I. Laboratories.

Diluents. In all cases except for the trypanostatic test *in vitro*, the diluent consisted of modified Alsever's solution containing 10% of horse serum (Soltys, 1957*b*).

The trypanostatic effect of suramin and antrycide on 'M' and 'R' variants was tested in a medium containing blood and used for growing some viruses (Dunne, Luedke, Reich & Hokanson, 1957). Preliminary experiments with the medium alone showed that about 80% of the trypanosomes survive in the medium for 3–4 days, but attempts to grow *T. brucei* in this medium failed.

This medium consisted of the following substances: defibrinated horseblood, 100 ml.; dihydrostreptomycin, 10 mg.; crystalline sodium penicillin, 1000 units; Tyrode's basic salt solution, 100 ml. 10 ml. of medium was placed into each of a series of 8 oz. Pyrex bottles and, after adding various concentrations of suramin, the bottles were inoculated with trypanosomes and incubated for 18 hr. at room temperature. At the end of this period the trypanosomes were examined microscopically for their motility and for infectivity by inoculation into mice.

EXPERIMENTAL

The effect of suramin and antrycide in vitro *on the infectivity for mice of* Trypanosoma brucei *variants 'M' and 'R'*

In the first phase of this investigation it was necessary to find whether the 'M' and 'R' strains possessed the same sensitivity to drugs when they were exposed to drugs *in vitro* and then injected into mice.

One series of flasks containing 10 ml. of Dunne's medium and various concentrations of suramin were inoculated with *T. brucei* 'M' variant and another with *T. brucei* 'R' variant. The number of trypanosomes in each flask was approximately 20,000 organisms per millilitre. Media without drugs were used as controls. The mixtures were left at room temperature for 18 hr., after which the trypanosomes, suspended in various concentrations of drugs, and the controls, were examined for their motility, and 0·5 ml. of each mixture was inoculated intraperitoneally into two mice to test its infectivity.

Similar experiments were carried out with antrycide sulphate. The results of these experiments are summarized in Tables 1 and 2. These tables show that the antibody-resistant strain 'R' is less sensitive to suramin and antrycide than the antibody-sensitive strain 'M'. While the antibody-sensitive strain was non-infectious to mice after exposure to 0·004 mg./ml. of either of the drugs for 18 hr., the antibody-resistant strain needed 50 times this concentration (0·2 mg./ml. of each drug) to render the strain non-infectious to mice. As both variants were

Table 1. *The effect of* in vitro *exposure of two variants of* Trypanosoma brucei *to suramin upon infectivity for mice*

Strain	No. of trypanosomes per ml.	Concentration of the drug (mg./ml.)	Period of exposure (hr.)	Fate of mice inoculated after 18 hr. exposure to the drug	The minimal concentration of the drug having trypanostatic effect (mg./ml.)
Variant 'M'	20,000	0·0001	18	Infected, incubation 2 days	
		0·001	18	Infected, incubation 5 days	0·004
	20,000	0·004	18	Not infected	
		0·02	18	Not infected	
		0·2	18	Not infected	
Variant 'R'	20,000	0·00025	18	Infected, incubation 2 days	
		0·001	18	Infected, incubation 3 days	
	20,000	0·004	18	Infected, incubation 10 days	0·2
		0·02	18	Infected, incubation 16 days	
		0·2	18	Not infected	

Table 2. *The effect of* in vitro *exposure of two strains of* Trypanosoma brucei *to antrycide sulphate upon infectivity for mice*

Strain	No. of trypanosomes per ml.	Concentration of the drug (mg./ml.)	Period of exposure (hr.)	Fate of mice inoculated after 18 hr. exposure to the drug	The minimal concentration of the drug having trypanostatic effect (mg./ml.)
Variant 'M'	20,000	0·00025	18	Infected, incubation 2 days	
		0·0001	18	Infected, incubation 5 days	0·004
		0·004	18	Not infected	
		0·02	18	Not infected	
		0·2	18	Not infected	
Variant 'R'	20,000	0·00025	18	Infected, incubation 3 days	
		0·001	18	Infected, incubation 3 days	
		0·004	18	Infected, incubation 5 days	0·2
		0·02	18	Infected, incubation 12 days	
		0·2	18	Not infected	

motile after exposure for 18 hr. to suramin and antrycide in all concentrations of drugs used in this experiment, it appears that the drugs in these concentrations have only trypanostatic activity. Thus the trypanosomes were not killed but were rendered more sensitive to the defensive mechanism of ths host.

The therapeutic effect of suramin and antrycide tested in mice infected with the antibody-sensitive variant 'M' and with the antibody-resistant variant 'R'

After the previous experiments which clearly demonstrated the difference in the sensitivity to trypanostatic drugs between the normal strain 'M' and the antibody-resistant strain 'R' *in vitro*, it was interesting to find whether the same difference would occur *in vivo*. A group of twelve mice were infected with the 'M' variant and another group of twelve were infected with the antibody-resistant variant 'R'. Each group was subdivided into four smaller groups consisting of three mice. These

Table 3. *The therapeutic effect of suramin on mice infected with two variants of* Trypanosoma brucei

Strain	No. of mice infected	Dosage of drug (mg.)	Observations and fate of mice	The minimal therapeutic dose (mg.)
Variant 'M'	3	0·01	All mice died within 6 days	
	3	0·05	All mice died within 14 days	0·1
	3	0·1	All mice survived	
	3	0·2	All mice survived	
Variant 'R'	3	0·01	All mice died within 8 days	
	3	0·05	All mice died within 14 days	
	3	0·1	All mice died within 21 days	0·3
	3	0·2	All mice died within 29 days	
	3	0·3	All mice survived	

were treated with doses of suramin ranging from 0·01 to 0·3 mg. A similar experiment was carried out on two other groups of twelve mice using antrycide sulphate. The treated animals were examined for the presence of trypanosomes in the blood for a period of 42 days. Results of these experiments are summarized in Tables 3 and 4. Minimal therapeutic dose signifies no relapse during observations over 42 days.

It appears from Tables 3 and 4 that the antibody-resistant strain needs three times the concentration of drugs required by the antibody-sensitive strain in order to stop the further development of trypanosomes in mice. This suggested that the curative dose for the normal strain would become subcurative for antibody-resistant strains and possibly would encourage the strain to become drug resistant.

Table 4. *The therapeutic effect of antrycide sulphate on mice infected with two variants of* Trypanosoma brucei

Strain	No. of mice infected	Dosage of drug after infection (mg.)	Fate of mice	The minimal therapeutic dose (mg.)
Variant 'M'	3	0·01	All mice died within 4 days	0·1
	3	0·05	All mice died within 10 days	
	3	0·1	All mice survived	
	3	0·3	All mice survived	
Variant 'R'	3	0·01	All mice died within 5 days	0·3
	3	0·05	All mice died within 8 days	
	3	0·1	All mice died within 28 days	
	3	0·2	All mice died within 28 days	
	3	0·3	All mice survived	

The prophylactic effect of suramin and antrycide tested in rabbits against Trypanosoma brucei '*M*' *and* Trypanosoma brucei '*R*'

Suramin

A group of twenty-four rabbits were inoculated intravenously with 50 mg. of suramin. After various periods of time (2, 3, 4 and 5 months), three of the rabbits were infected with *T. brucei* 'M' and the same number with *T. brucei* 'R'. The results of this experiment are summarized in Table 5.

It appears from the results shown in Table 5 that rabbits injected prophylactically with 50 mg. of suramin can resist *T. brucei* 'M' for a period of 4 months, but the strain of *T. brucei* 'R' for only 2 months. These results are in agreement with results of experiments on the trypanostatic effect *in vitro* and the therapeutic effect in mice, indicating that the antibody-resistant variant 'R' can withstand a higher concentration of drugs than the normal strain 'M'. Thus when the level of suramin in the animal is declining after 2 months, the amount present is not sufficient to control the antibody-resistant strain, but is still sufficient to control the normal strain 'M', which, unlike strain 'R', is not only affected by the drug but also by the natural defence forces of the host.

Antrycide pro-salt

A similar experiment to that with suramin was carried out with antrycide-pro-salt. The results are summarized in Table 6, and show that rabbits treated prophylactically with 15 mg. of antrycide pro-salt resisted infection with *T. brucei* 'M' for a period of 2 months, but rabbits infected with the 'R' variant developed trypanosomiasis one month after the prophylactic infection of the drug.

Table 5. *Prophylactic effect of suramin against two variants of* Trypanosoma brucei

No. of rabbit	Dose of the drug (mg.)	Interval between injection of the drug and injection of trypanosomes (months)	Fate of rabbit	Result
			Strain variant 'M'	
M 1	50	2	Survived	Prophylactic effect for a period of 4 months
M 2	50	2	Survived	
M 3	50	2	Survived	
M 4	50	3	Survived	
M 5	50	3	Survived	
M 6	50	3	Survived	
M 7	50	4	Survived	
M 8	50	4	Survived	
M 9	50	4	Survived	
M 10	50	5	Died 138 days after infection	
M 11	50	5	Died 90 days after infection	
M 12	50	5	Survived	
			Strain variant 'R'	
R 13	50	2	Survived	Prophylactic effect for a period up to 2 months only
R 14	50	2	Survived	
R 15	50	2	Died 107 days after infection	
R 16	50	3	Died of trypanosomiasis 55 days after infection	
R 17	50	3	Died of trypanosomiasis 42 days after infection	
R 18	50	3	Died of trypanosomiasis 49 days after infection	

The effect of immune bodies on the suramin-resistant variant 'D.F.'

In order to find whether the drug-resistant strain was also an antibody-resistant strain, the following experiments were carried out. A strain of *T. brucei* 'M' which was made suramin fast was able to resist doses of 10 mg. of the drug while the minimum effective dose for the normal strain was 0·1 mg. The suramin-fast strain 'D.F.' was tested in parallel with *T. brucei* strain 'M' against rabbit serum containing neutralizing antibodies. It was found that the suramin-fast strain was antibody sensitive and was neutralized by the antiserum in the same titre (1:160) as the 'M' variant. It appears from these experiments that it is possible to develop a drug-resistant strain which is not at the same time antibody resistant. When strain 'D.F.' was tested against antrycide sulphate, it failed to show any resistance to this drug.

Table 6. *Prophylactic effect of antrycide pro-salt against two variants of* Trypanosoma brucei

No. of rabbit	Dose of the drug (mg.)	Interval between injection of the drug and injection of trypanosomes (months)	Fate of rabbit	Result
			Strain variant 'M'	
M 19	15	1	Survived	Prophylactic effect for a period between 1 and 2 months
M 20	15	1	Survived	
M 21	15	1	Survived	
M 22	15	2	Died of trypanosomiasis 138 days after infection	
M 23	15	2	Died of trypanosomiasis 144 days after infection	
M 24	15	2	Survived	
M 25	15	3	Died of trypanosomiasis 55 days after infection	
M 26	15	3	Died of trypanosomiasis 42 days after infection	
M 27	15	3	Died of trypanosomiasis 61 days after infection	
			Strain variant 'R'	
R 28	15	1	Died of trypanosomiasis 35 days after infection	No prophylactic effect against 'R' variant
R 29	15	1	Died of trypanosomiasis 42 days after infection	
R 30	15	1	Died of trypanosomiasis 44 days after infection	
R 31	15	2	Died of trypanosomiasis 31 days after infection	
R 32	15	2	Died of trypanosomiasis 28 days after infection	
R 33	15	2	Died of trypanosomiasis 32 days after infection	

Two controls infected with the 'M' strain died after 52 and 42 days respectively.
Two controls infected with the 'R' strain died after 35 and 27 days respectively.

DISCUSSION

The idea that the immune bodies and natural mechanism of defence play an important role in chemotherapy has been discussed since Ehrlich began the study of chemotherapy and introduced the theory of 'ictus immunisatorius'. Ehrlich & Gonder (1920), Browning (1927) and Jancsó & Jancsó (1934) assumed that a definite sterilization of the infected animal is in most circumstances due to a combined mechanism of the chemical substance and the defensive mechanism of the host. This assumption had been confirmed by the present experiments, which showed that a variant of *T. brucei* which was exposed to antibodies for some time and became an antibody-tolerant or antibody-resistant strain, was less sensitive to suramin and antrycide than a strain which had never been exposed to antibodies. Experiments on the *in vitro* effect of drugs upon the infectivity of these two variants

for mice showed clearly that the antibody-resistant strain needed 50 times more drugs than the antibody-sensitive strain in order to become non-infectious to mice. On the other hand, therapeutic experiments showed that only three times as much drug was required to eliminate the antibody-resistant strain from mice in comparison with the antibody-sensitive 'M' variant. More striking differences between these variants were shown in experiments on the prophylactic effect of suramin and antrycide. Animals treated prophylactically with suramin or antrycide resisted challenge with the antibody-sensitive strain for a much longer period than animals challenged with the antibody-resistant strain. These experiments showed without any doubt that in chemotherapy and chemoprophylaxis with trypanostatic agents the natural mechanism of defence and immune bodies play an important part.

Since the natural defence forces play an important part in the chemotherapy and chemoprophylaxis of trypanosomiasis it is important to ascertain whether the development of drug-fast strains in animals is influenced by the development of antibody resistance with a consequent exclusion of the natural mechanism of defence. Jancsó & Jancsó (1935) and Hawking (1939) produced suramin-resistant strains in 4 months in animals in which the defence forces were upset by splenectomy and blockading of the reticulo-endothelial system (R.E.S.) with colloidal copper, whereas in normal animals it took more than 12 months (Yorke, Murgatroyd & Hawking, 1932). It is, however, doubtful whether in nature the development of drug resistance goes through the same long procedure as when artificially produced under laboratory conditions by many workers in the past. Although it is possible experimentally to develop drug resistance without antibody resistance it seems most unlikely that such a procedure takes place in nature. In field cases most of the drug-resistant strains are most probably also antibody-resistant strains, as they will have been exposed to antibodies for some time before treatment of the affected animal begins.

The idea that the host participates in the phenomenon of drug resistance, suggested by early workers (Mesnil & Brimont, 1908; Moore, Nierenstein & Todd, 1908; Jancsó & Jancsó, 1934), has been confirmed by experiments discussed in this paper. Experiments of Yorke & Hawking (1932), however, failed to confirm the observations of early workers, and came to the conclusions that the host represents only a passive medium and the protective powers of the host are not involved in the genesis of drug resistance. It is possible, however, that the latter workers were dealing with drug-resistant strains which were not at the same time antibody resistant, as they used for their experiments mostly mice and rats, animals in which infection is short-lasting and the development of antibodies is poor or cannot be demonstrated. In Mesnil & Brimont's (1908) experiment a strain was isolated from a horse which was treated with atoxyl and received 34 g. of the drug within a period of 28 days. Therefore there is no doubt that this strain during 28 days in a horse, an animal which produces good antibodies, first became an antibody resistant.

It appears from the experiments of previous workers and from the writer's experiments that it is much easier to obtain a drug-resistant strain by removing

the defensive mechanism of the host by (*a*) splenectomy and blockading the reticulo-endothelial system (Jancsó & Jancsó, 1934; Hawking, 1939), or by (*b*) producing first antibody-resistant strains (Soltys, 1957*b*) which are then not affected by the defensive system of the host. There is no doubt that the latter method occurs in nature, and therefore in all tests of the efficiency of chemotherapeutic drugs, antibody-resistant strains (obtained by passage in rabbits or in other animals in which the infection is sufficiently lasting to expose the strain for some time to antibodies) should be used, as well as antibody-sensitive strains frequently passaged through mice.

SUMMARY

Antibody-resistant strains are less sensitive to suramin and antrycide than antibody-sensitive strains. When living trypanosomes were exposed to suramin and antrycide *in vitro*, antibody-resistant strains needed 50 times more drugs than antibody-sensitive trypanosomes in order to make them non-infectious to mice. In therapeutic experiments in mice the minimal therapeutic dose of drugs for antibody-sensitive strains was 0·1 mg. but for resistant strains it was 0·3 mg./20 g. mice. Rabbits treated prophylactically with suramin resisted infection with the antibody-sensitive strain for a period of 4 months, but failed to resist infection with the antibody-resistant strain after 2 months.

Rabbits treated prophylactically with antrycide pro-salt, resisted infection with antibody-sensitive strains for a period of 2 months, but failed to resist infection with the antibody-resistant strain even 1 month after injection with the drug. Although trypanosomes can become drug resistant without being antibody resistant it is suggested that, under natural conditions, drug-resistant strains in animals and man develop from antibody-resistant strains, particularly when trypanostatic drugs are used. It is suggested in conclusion from these experiments that strains of trypanosomes which are exposed for some time to antibodies and become antibody resistant after passage through animals like rabbits, as well as those strains frequently passaged through mice, should be used in all tests for the efficiency of chemotherapeutic drugs.

REFERENCES

Browning, C. H. (1927). Biological principles in immunity. *Brit. Med. J.* **2**, 978.

Dunne, H. W., Luedke, A. I., Reich, C. V. & Hokanson, J. F. (1957). The *in vitro* growth of hog cholera virus in cells of peripheral blood. *Amer. J. Vet. Res.* **28**, 502.

Ehrlich, P. & Gonder, R. (1920). Experimentelle chemotherapie. *Prowazeks Handb. Patholog. Protozoen*, **2**, 752.

Hawking, F. (1939). Contribution on the mode of action of Germanin (Bayer 205). *Ann. Trop. Med. Parasit.* **33**, 13.

Jancsó, N. & Jancsó, H. (1934). The role of the natural defence forces in the evolution of the drug resistance of trypanosomes. I. A method for the exclusion of the natural defence mechanisms from chemotherapeutic processes. *Ann. Trop. Med. Parasit.* **28**, 419.

Jancsó, N. & Jancsó, H. (1935). The role of the natural defence forces in the evolution of the drug resistance of trypanosomes. II. The rapid production of germanin-fast *T. brucei* strains in animals with paralysed defence. *Ann. Trop. Med. Parasit.* **29**, 95.

Mesnil, F. & Brimont, E. (1908). Sur les propriétés des races de trypanosomes résistantes aux médicaments. *Ann. Inst. Pasteur*, **22**, 856.

MOORE, B., NIERENSTEIN, M. & TODD, J. L. (1908). Notes on the effects of therapeutic agents on trypanosomes in respect of (*a*) acquired resistance of the parasites to the drug and (*b*) changes in virulence of the strains after escape from the drug. *Ann. Trop. Med. Parasit.* **2**, 221.

POLGE, C. & SOLTYS, M. A. (1957). Preservations of trypanosomes in the frozen state. *Trans. R. Soc. Trop. Med. Hyg.* **51**, 519.

SOLTYS, M. A. (1957*a*). Immunity in trypanosomiasis. I. Neutralization reaction. *Parasitology*, **47**, 375.

SOLTYS, M. A. (1957*b*). Immunity in trypanosomiasis. II. Agglutination reaction with African trypanosomes. *Parasitology*, **47**, 390.

YORKE, W. & HAWKING, F. (1932). Studies in chemotherapy. VII. Is the resistance of a drug-fast trypanosome modified by transference to a different species of vertebrate host? *Ann. Trop. Med. Parasit.* **26**, 215.

YORKE, W., MURGATROYD, F. & HAWKING, F. (1932). Studies in chemotherapy. VIII. Comparison of strains of *T. rhodesiense* made resistant to various arsenicals and antimonials to Bayer 205 and to acriflavine respectively. *Ann. Trop. Med. Parasit.* **26**, 577.

(*MS. received for publication* 11. IV. 1958—Ed.)

MONOGENEA OF INDIAN FRESHWATER FISHES

VIII. *SPROSTONIA*, A NEW GENUS OF FRESHWATER TETRAONCHINAE, WITH THE DESCRIPTIONS OF FOUR NEW SPECIES, FROM THE GILL FILAMENTS OF FISHES, FROM LUCKNOW

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(With 21 Figures in the Text)

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I. INTRODUCTION

Our knowledge of Indian monogenetic trematodes from the freshwater fishes is limited to about a dozen forms. The earliest record was established by Price (1938), who described *Dactylogyrus moorthyi* sp.nov., from Mysore. Thapar (1948) described *Paradactylogyrus* gen.nov., and Jain (1952*a*, *b*, 1955, 1957*a–c*), in a series of papers, described *Haplocleidus gomtius* sp.nov., *Thaparocleidus wallagonius* gen.nov., sp.nov., *Urocleidus notopterus* sp.nov., *Mizelleus indicus* gen.nov., sp.nov., *Dactylogyrus multispiralis* sp.nov., and three new species of the genus *Neodactylogyrus*, namely *N. calbasi*, *N. indicus* and *N. cotius*, from Lucknow. Recently, Baugh (1957) has erected a new genus, *Neogyrodactylus*, from West Bengal.†

The author took up a systematic investigation of the trematodes parasitic in the gill filaments of freshwater fishes available in the Lucknow region. It was found that most fishes carry heavy infestations of monogenetic trematodes. The present paper, which is the eighth in the series, describes a new genus based on four new forms of the subfamily Tetraonchinae (Family Dactylogyridae).

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† Please see postscript at the end of the paper.

II. MATERIALS AND METHODS

Fishes for the present investigation were purchased from the local fish market, Lucknow. The identification of the host was made from the papers of Shaw & Shebbeare (1937) and Chauhan & Ramakrishna (1953).

Mizelle's refrigeration technique (1936, 1938) was employed for the recovery of trematodes from the gill filaments. Hargis's (1952, 1953) chloretone technique had the additional advantage in saving time and labour and can be applied to fresh specimens, which are more transparent and more suitable for the study of taxonomic characters.

Measurements of all chitinized structures were made in glycerine mounts. Curved surfaces were measured across the line subtending the greatest arcs described by those structures, as suggested by Mizelle (1936, 1938). In all cases an attempt was made to give the range in variation of different structures by examining several trematodes.

III. *SPROSTONIA INDICA* GEN.NOV., SP.NOV.

Host: *Eutropiichthys vacha* (Ham.). Number of fishes examined: 24. Number of fishes found infected: 20. Number of trematodes studied: 10.

Stout elongated tetraonchids (Fig. 1), 0·55–0·69 mm. long, with width at the cephalic lobes 0·038 mm.; maximum body width 0·085 mm. Head organs in four pairs. Two pairs of eye-spots, one pair anterior and the other posterior, the latter with a large number of melanistic granules which are scattered with the slightest pressure of the coverglass. Pharynx, spherical, measuring 0·025 mm. in maximum diameter. Intestine, bifurcate, the crura being confluent posteriorly.

The haptor (Fig. 2) is 0·1–0·15 mm. long and 0·09–0·1 mm. wide. Its peduncle is short and 0·05–0·06 mm. wide. The armature of the haptor (Fig. 3) consists of two pairs of anchors, a pair of bars, a pair of supporting bars and seven pairs of hooks. Dorsal anchors are very large as compared to the ventral anchors, and each consists of a stout rounded base, a stout shaft and fine recurved points. The base has a small lateral projection, but there is no differentiation of superficial and deep root. There is a single wing attached to each dorsal anchor, located at the posterior end. Each dorsal anchor is 0·062–0·075 mm. long, and 0·013–0·017 mm. wide at the base. The dorsal bar is a straight transverse shaft, with a wide aperture in the middle and is 0·05–0·062 mm. long and 0·013 mm. in maximum width. The pair of supporting bars is situated at the base of the dorsal anchors. Each supporting bar is a stout conical body, rounded proximally and pointed distally, and is 0·032–0·036 mm. long. Each ventral anchor consists of a stout base, a shaft with a pair of rounded projections internally, and fine recurved points making an acute angle with the shaft. There is no clear differentiation of roots in the ventral anchors. No wing has been observed on the anchors. The two projections on each ventral anchor are unequal in size, the dorsal projection (Fig. 3, *d.p.*) being larger than the ventral one (Fig. 3, *v.p.*). Each ventral anchor is 0·048–0·057 mm. long and 0·017–0·021 mm. wide at the base. The ventral bar is a stout, transverse shaft, consisting of two pieces, the two transverse pieces being firmly articulated in the

middle. Near the point of articulation each piece shows a tooth-like projection, anteriorly. The ventral bar is 0·11–0·13 mm. long. The seven pairs of hooks are

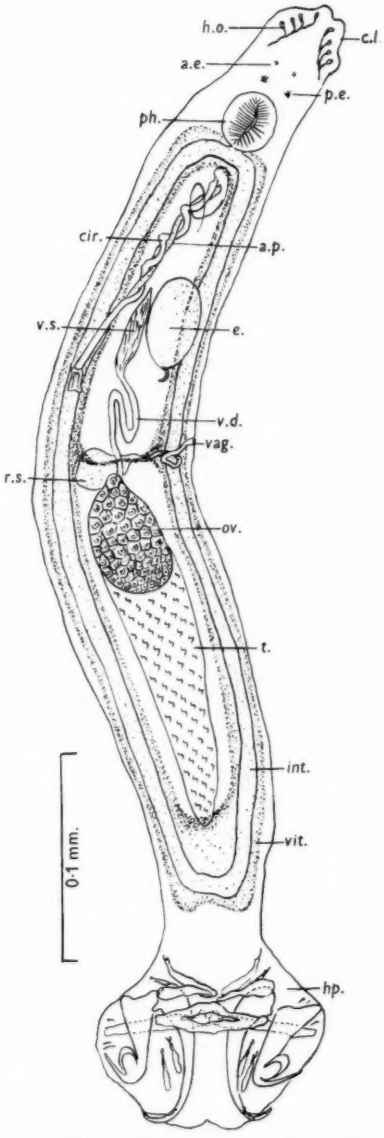


Fig. 1. *Sprostonia indica* gen.nov., sp.nov.; dorsal view of the worm.

all similar in shape, but differ in size. Each hook is crochet-shaped, consisting of an elongated base, an insignificant shaft and a small crochet-shaped portion. The dimensions of the hooks are: pair 1, 0·036–0·037 mm.; pair 2, 0·019–0·02 mm.;

pair 3, 0·012–0·013 mm.; pair 4, 0·012–0·013 mm.; pair 5, 0·011–0·012 mm.; pair 6, 0·027–0·029 mm.; and pair 7, 0·028–0·036 mm.

The testis (Fig. 1) is median, elongated and intercaecal, measuring 0·14–0·16 × 0·027–0·039 mm. It is continued into a spindle-shaped vesicula seminalis and a U-shaped vas deferens. The copulatory complex (Fig. 4) is very stout, consisting of a very long cork-screw-like cirrus, an elongated accessory piece and a coiled

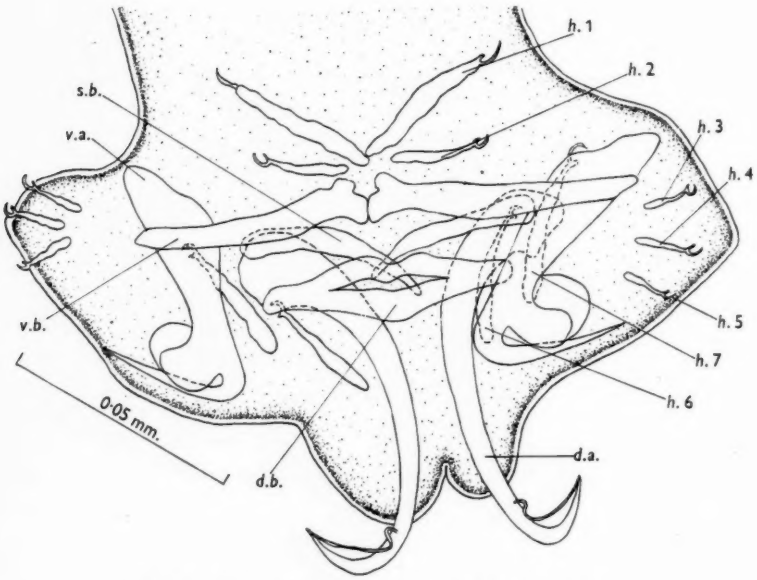


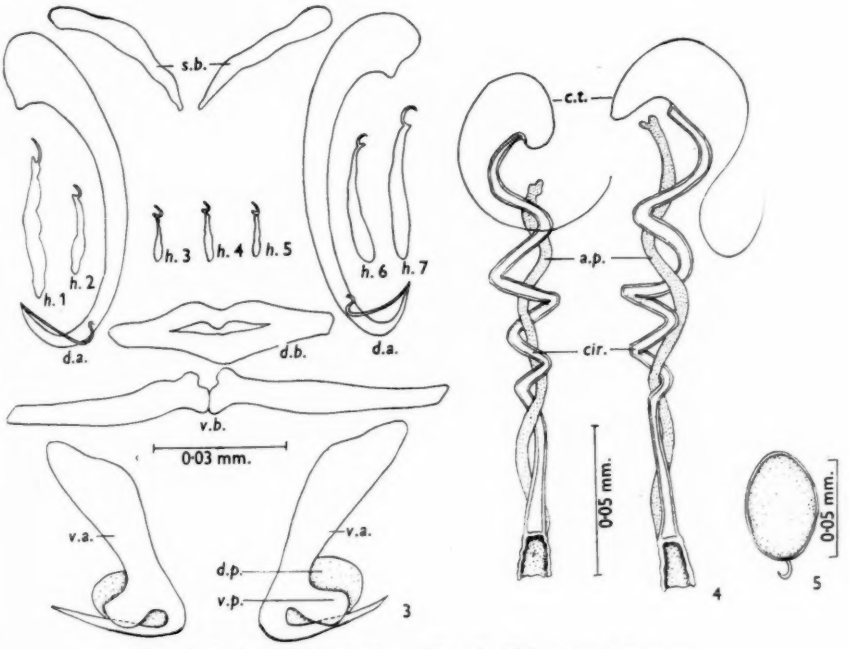
Fig. 2. *Sprostonia indica*; ventral view of haptor.

cirrhal thread. The cirrus and the accessory piece are firmly articulated at the base. The cirrus is a thick-walled, hollow tube, its body having three or four spiral turns around the accessory piece, it is 0·125–0·165 mm. long. The accessory piece lies parallel to the cirrus and is 0·1–0·125 mm. long. The cirrhal thread is a continuation of the cirrus. Prostate glands are absent. The ovary is small, pyriform, median and intercaecal, measuring 0·056–0·063 × 0·025–0·033 mm. and is filled with nucleated ova. The vagina (Fig. 1) is dextral, chitinized and has a short funnel-shaped mouth, a looped vaginal tube and a small pear-shaped receptaculum seminis. The single egg (Fig. 5) observed in some specimens is oval with a hook-like posterior projection; it measures 0·055 × 0·04 mm. The vitellaria extend from the pharynx to the caecal union, the vitelline ducts joining in the middle.

Discussion. The present form is undoubtedly a member of the subfamily Tetraonchinae Monticelli, 1903, because it has the two pairs of anchors and no squamodiscs. It is differentiated from all the known genera of this subfamily by the characteristic two-piece nature of the ventral bar. It shows some affinity with the genera *Urocleidus* Mueller, 1934, and *Cleidodiscus* Mueller, 1934, but

differs from *Urocleidus* in that the cirrus and accessory piece are firmly articulated at the base, while the position of the vagina differentiates it from *Cleidodiscus*.

It is evident, therefore, that the present form is different from all the known genera of Tetraonchinae. It is therefore assigned to a new genus *Sprostonia* gen. nov., named after Dr Nora G. Sproston, in appreciation of her monograph on the Monogenea.



Figs. 3–5. *Sprostonia indica*. Fig. 3. Elements of haptor. Fig. 4. Copulatory complex. Fig. 5. Egg.

IV. *SPROSTONIA WALLAGONIA* SP.NOV.

Host: *Wallagonia attu* (Bloch). Number of fishes examined: 54, of which 27 were found infected. Number of trematodes studied: 12.

Stout elongated tetraonchids (Fig. 6), 0·58–0·73 mm. long, the width at the cephalic lobes being 0·06 mm. and the maximum body width 0·1 mm. The cephalic region bears four pairs of head organs. There are two pairs of eye-spots, one anterior and the other posterior, the latter having a larger number of melanic granules. The pharynx is spherical, with a maximum diameter 0·028 mm. The intestine is bifurcate and the crura are confluent posteriorly. The outlines of the intestine are obliterated in some specimens by the dense vitellaria in that region.

The haptor (Fig. 7) is discoidal to somewhat hexagonal in shape, 0·085–0·13 mm. long and 0·055–0·1 mm. wide. The armature of the haptor (Fig. 8) consists of a pair of stout dorsal and a pair of smaller ventral anchors, dissimilar in shape and size, a dorsal and a ventral bar, a pair of stout supporting bars and six pairs of

hooks. Each dorsal anchor consists of a rounded base, a stout shaft and a fine recurved point. The base has a shallow groove, but there is no clear differentiation of a superficial and a deep root. Each dorsal anchor is provided with a single wing,

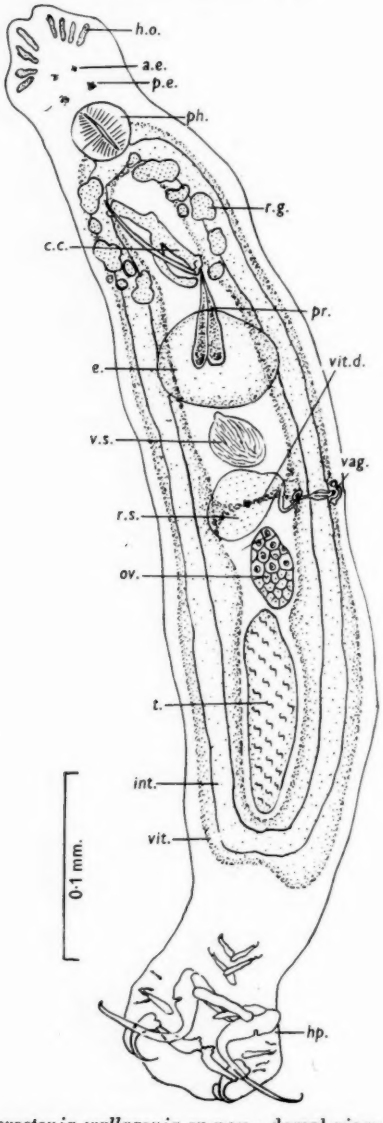
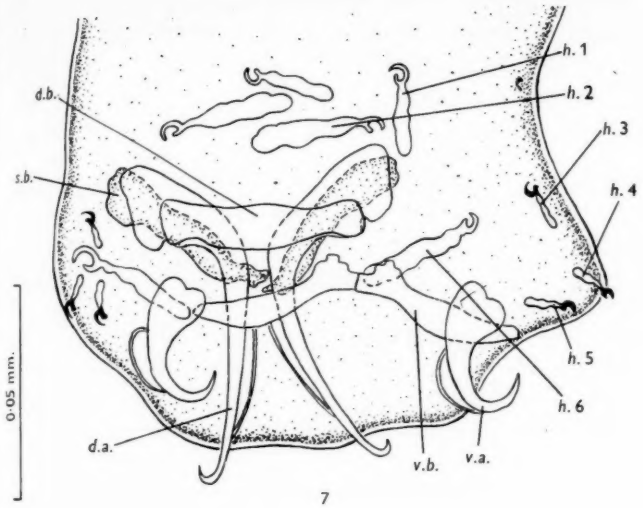


Fig. 6. *Sprostonia wallagonia* sp.nov.; dorsal view of worm.

which does not reach up to the points. Each dorsal anchor is 0·07–0·078 mm. long and the base is 0·02 mm. wide. The dorsal bar is a stout, transverse shaft, 0·044–0·049 mm. long and 0·01 mm. in maximum thickness. The pair of supporting bars



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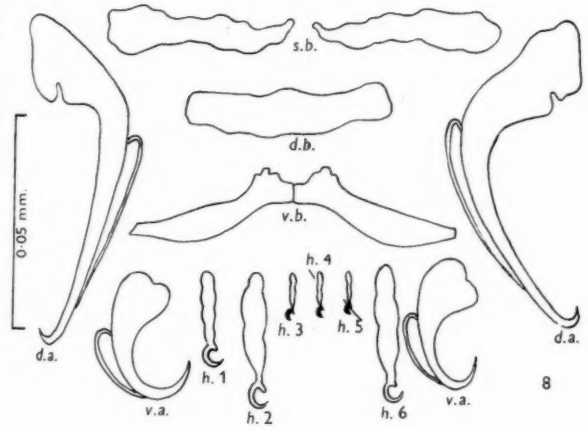

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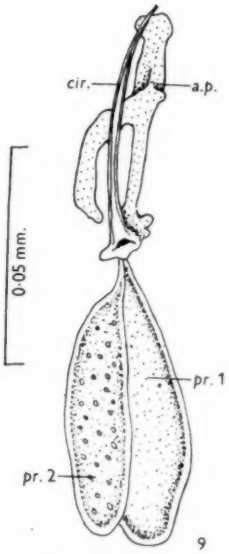

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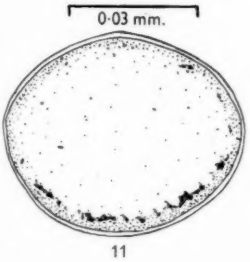

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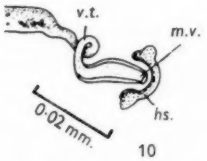

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Figs. 7–11. *Sprostonia wallagonia*. Fig. 7. Ventral view of haptor. Fig. 8. Elements of haptor. Fig. 9. Copulatory complex. Fig. 10. Vagina. Fig. 11. Egg.

are situated at the bases of the dorsal anchors. Each supporting bar is a conical body with a rounded proximal, and a pointed distal, end, and is 0·044–0·047 mm. long. In the study of fresh material the supporting bars form a V, with arms wide apart. Each ventral anchor consists of a rounded base, a shaft and fine recurved points. The base of the ventral anchors is slightly bifurcate, consisting of a short rounded deep and a similar superficial root. Each anchor which is provided with a single wing is 0·028–0·031 mm. long, 0·012–0·014 mm. wide at the base. The ventral bar is yoke-shaped, with a shallow groove anteriorly and a wide groove posteriorly. It is a two-piece bar, and is 0·075–0·079 mm. long. Six pairs of hooks present, all similar in shape but dissimilar in size. Each hook consists of swollen base, a short shaft and a crochet-shaped portion. The dimensions of the hooks are: pair 1, 0·021–0·022 mm.; pair 2, 0·03–0·032 mm.; pair 3, 0·01–0·013 mm.; pair 4, 0·009–0·012 mm.; pair 5, 0·009–0·012 mm.; and pair 6, 0·032–0·034 mm.

The testis (Fig. 6) is median, oblong and intercaecal in position, measuring 0·1–0·121 × 0·021–0·029 mm. The vesicula seminalis is short, pyriform and lies medially. The copulatory complex (Fig. 9) consists of a cirrus and an accessory piece both articulated firmly at the base. The cirrus is stout, thick-walled, terminated in a fine point, and is 0·064–0·067 mm. long. The accessory piece has the shape of an inverted Y and is 0·058–0·06 mm. long. Two prostate glands are present at the base of the cirrus, one of these (*pr.* 1) is filled with a hyaline fluid and the other (*pr.* 2) is filled with highly granular fluid. The ovary is oval, pretesticular and median, measuring 0·04–0·047 × 0·011–0·014 mm. The vagina (Fig. 10) is dextral, highly chitinized and has a horse-shoe-shaped support at the mouth. The vaginal tube has one loop and leads into a large, pear-shaped receptaculum seminis. The single egg (Fig. 11) observed in some specimens is thin-shelled, oval, and measures 0·06 × 0·048 mm. The vitellaria extend from the pharynx to the caecal union, the vitelline ducts join in the middle, near the receptaculum seminis. The so-called reproductive glands observed by Jain (1957 *a*) in *Mizelleus indicus* are present in groups around the region of the copulatory complex; they stain brightly with Ehrlich's haematoxylin.

Discussion. The present form, while agreeing in all essential respects with the genotype *Sprostonia indica* gen.nov., sp.nov., differs from it in these characteristics: (1) the shape of the cirrus and the accessory piece, (2) the absence of projections on the ventral anchors, (3) the conspicuously smaller size of the ventral bar, and (4) the shape of the dorsal bar.

The present form thus differs from the genotype and it is therefore assigned to the new species *Sprostonia wallagonia* sp.nov., named after the host.

V. *SPROSTONIA GOMTIA* SP.NOV.

Host: *Eutropiichthys vacha* (Ham.). Number of fishes examined: 24, of which 4 were found infected. Number of trematodes studied: 6.

Slender elongated tetraonchids (Fig. 12), 0·7–0·82 mm. long, the width at the cephalic lobes being 0·06–0·07 mm. and the maximum body width 0·1–0·12 mm. The anterior end, differentiated into two pairs of cephalic lobes, bears three pairs

of head organs. There are two pairs of eye-spots, one anterior and one posterior, the latter having a rather larger number of melanic granules. The pharynx is oval, measuring 0·03 × 0·035 mm. The intestine is bifurcate and the crura are confluent posteriorly.

The haptor (Fig. 13) is discoidal to somewhat hexagonal, 0·1–0·12 mm. long and 0·12–0·16 mm. wide. Its peduncle is long and stout. The armature of the haptor consists of a pair of stout dorsal anchors, a pair of small ventral anchors, a dorsal and a ventral bar, a pair of supporting bars, and seven pairs of hooks with a conspicuous central onchium. Each dorsal anchor (Fig. 14) consists of a stout base, a long shaft and a fine, recurved point. The base is rounded with a short, blunt, anterior projection. There is no differentiation of a superficial and a deep root. Each anchor is provided with a single wing, which does not reach up to the points. Each dorsal anchor is 0·07–0·076 mm. long and 0·02 mm. wide at the base. The dorsal bar is a straight, transverse shaft with a large aperture in the middle. It is 0·06–0·063 mm. long and its maximum width is 0·015 mm. Supporting bars are situated at the bases of the dorsal anchors; each has a rounded base, a long shaft and fine point, and measures 0·043–0·045 mm. in length. Each ventral anchor consists of a stout base, a shaft bearing a pair of projections, and fine recurved points. The base has a short, conical, superficial and inconspicuous deep root. Each ventral anchor has a single wing, not reaching up to the point; it is 0·037–0·042 mm. long and its width at the base is 0·015 mm. The width of the ventral anchor at the first projection is 0·006–0·01 mm., and at the second 0·01–0·014 mm. The ventral bar is a straight, transverse shaft, composed of two pieces and 0·11–0·125 mm. long. The seven pairs of hooks are all similar in shape but differ in size. They have the typical tetraonchid type of arrangement. Each hook is crochet-shaped, consisting of a stout oblong base, an insignificant shaft and a crochet-shaped portion. The dimensions of the hooks are as follows: pair 1, 0·029–0·03 mm.; pair 2, 0·033–0·036 mm.; pair 3, 0·011–0·013 mm.; pair 4, 0·011–0·014 mm.; pair 5, 0·009–0·011 mm.; pair 6, 0·034–0·035 mm.; and pair 7, 0·043–0·044 mm.

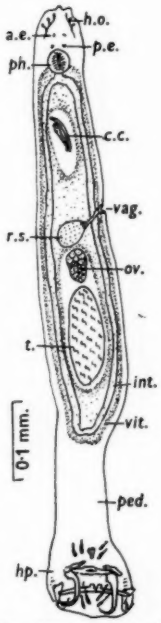
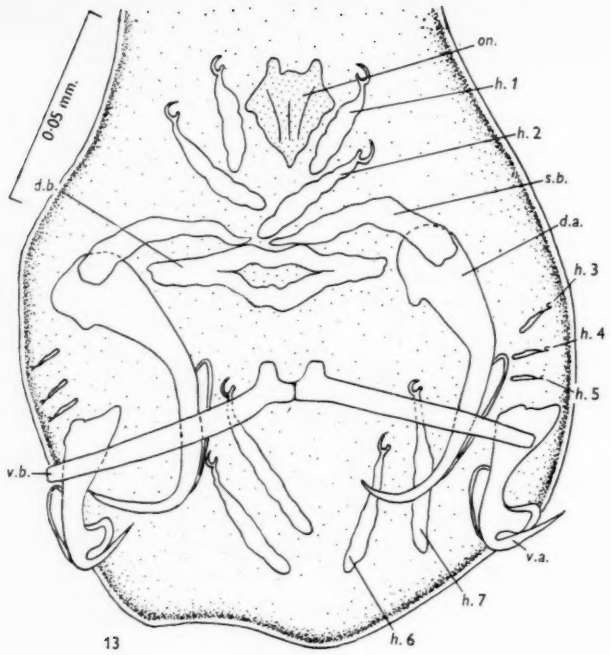


Fig. 12. *Sprostonia gomtia* sp.nov.; dorsal view of worm.

There is a central leaf-like plate situated between the first pair of hooks (Figs. 13, 14), which appears to be described here for the first time in a member of the subfamily Tetraonchinae. It is named 'onchium', adopting the term given by Thapar (1948) to a central haptoral structure, which he observed in *Paradactylogyrus catlaius*, a new member of the family Dactylogyrinae. The onchium in Thapar's form was elongated and stick-like, while in the present form it is large and leaf-like. It measures 0·024–0·027 mm. in length and 0·02–0·022 mm. in width.

The testis (Fig. 12) is median, elliptical and intercaecal in position, measuring 0·13–0·15 × 0·045–0·052 mm. The vesicula seminalis is not clear. The copulatory



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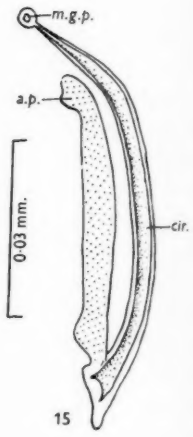

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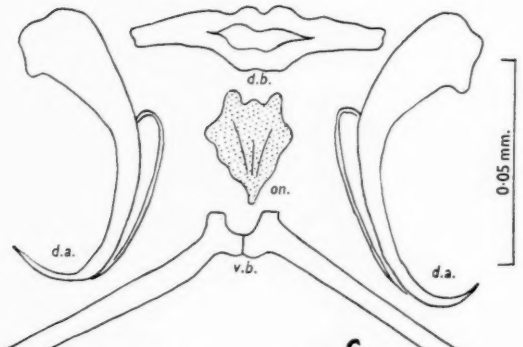

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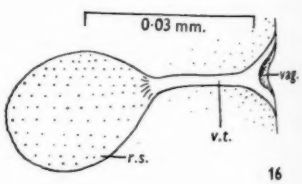

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Figs. 13–16. *Sprostonia gomtia* sp.nov. Fig. 13. Ventral view of haptor. Fig. 14. Elements of haptor. Fig. 15. Copulatory complex. Fig. 16. Vagina.

complex (Fig. 15) consists of a cirrus and an accessory piece, both firmly articulated together at the base. The cirrus is stout, elongated thick-walled and is 0·08–0·084 mm. long. The accessory piece is a longitudinal shaft, running parallel to the cirrus and is 0·05–0·052 mm. long. The prostate glands are not clear. The ovary is pretesticular, roughly oval, intercaecal in position and measures 0·04–0·043 × 0·026–0·035 mm. The vagina (Fig. 16) is dextral, only slightly chitinized, and opens into an oval receptaculum seminis by a short vaginal canal. No egg has been observed in the specimens studied. The vitellaria extend from the pharynx to the caecal union; the vitelline ducts are not clear.

Discussion. The present form is assigned to the genus *Sprostonia* gen.nov., because the ventral bar is composed of two pieces, the cirrus articulates with the accessory piece at the base, and the vagina is dextral. It differs, conspicuously, from the genotype *S. indica* and *S. wallagonia* sp.nov., in the presence of a central onchium in the haptor.

Further, from *S. indica*, the present form can be distinguished by (1) the shape of the cirrus, (2) the shape of the accessory piece, (3) the shape of the ventral bar, and (4) the smaller size of the ventral anchors as compared with the dorsal anchors.

From *S. wallagonia* sp.nov., the present form can be distinguished by (1) the presence of conspicuous projections on the ventral anchor shafts, (2) the shape of the dorsal bar; and (3) the shape of the accessory piece.

It is evident, therefore, that the present form is distinct and hence assigned to the new species *Sprostonia gomtia* sp.nov., named after the local river, Gomti.

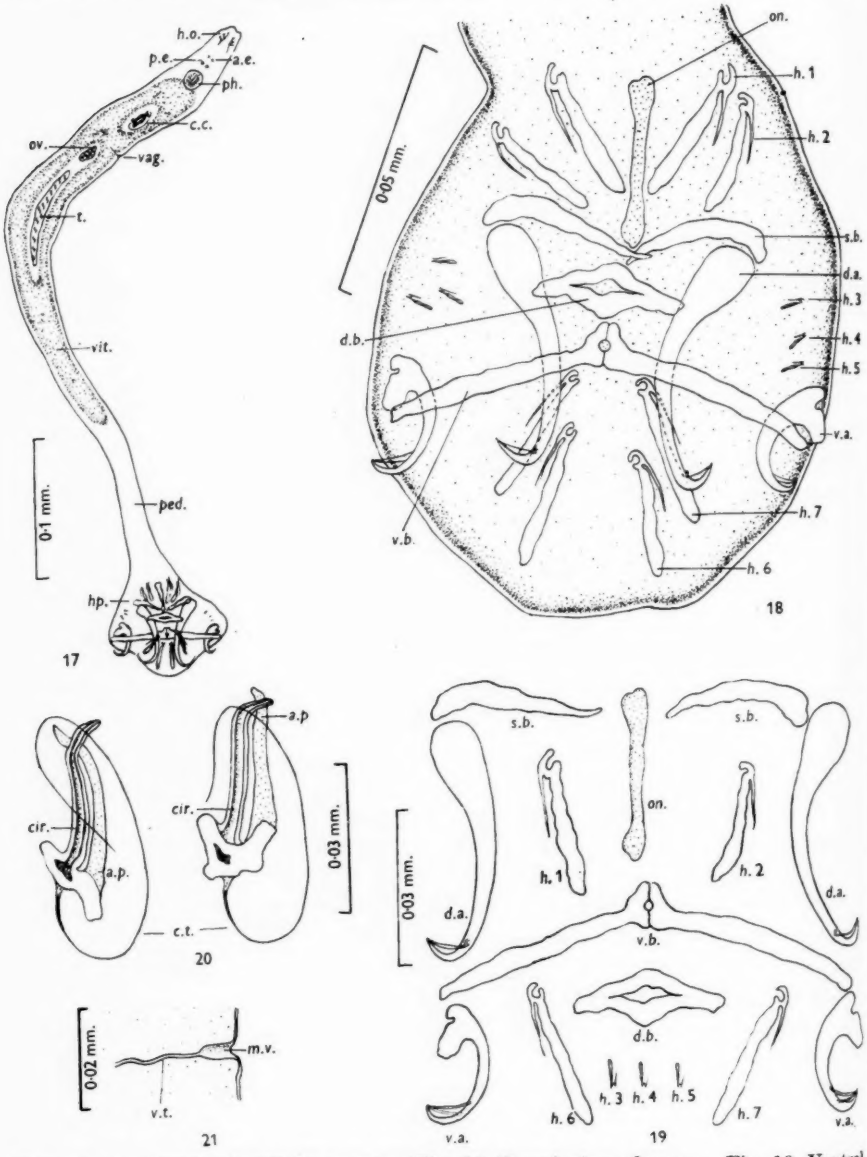
VI. *SPROSTONIA ASIATICA* SP.NOV.

Host: *Callichrous pabda* Hamilton. Number of fishes examined: 15. Number of fishes found infected: 3. Number of trematodes studied: 5.

Slender, elongated tetraonchids (Fig. 17), measuring 0·52–0·64 mm. in length, the width at the cephalic lobes being 0·022 mm., and the maximum body width 0·042 mm. There are three pairs of poorly developed head organs. There are two pairs of eye-spots, one anterior and one posterior, the latter having a larger number of melanic granules. The pharynx measures 0·012 × 0·01 mm. The outlines of the intestine could not be worked out because of the large amount of vitellaria spread in that region.

The haptor (Fig. 18) is 0·09–0·11 mm. long and 0·07–0·09 mm. wide. Its peduncle is long and slender. The armature of the haptor (Fig. 19) consists of a pair of stout, dorsal anchors, smaller ventral anchors, a dorsal and a ventral bar, a central onchium and seven pairs of hooks. Each dorsal anchor consists of a rounded swollen base, a shaft and fine recurved points. The base is rounded and there is no differentiation of superficial and deep root. The wings on the dorsal anchors are much reduced and are restricted to the most distal part of the point. Each dorsal anchor is 0·048–0·051 mm. long, and 0·01 mm. wide at the base. The dorsal bar is a straight transverse shaft, with a large aperture in the middle and is 0·028–0·031 mm. long. The supporting bars are situated at the bases of the dorsal anchors, each being

0·028–0·036 mm. long. Each ventral anchor consists of a stout base, differentiated into an expanded superficial and a short, blunt deep root, a shaft and a recurved point. The wings on the ventral anchors are also reduced, double in number, and reach up to the points. Each ventral anchor is 0·023–0·025 mm. long, the width of the base being 0·01–0·012 mm. The ventral bar is a straight, transverse shaft.



Figs. 17–21. *Sprostonia asiatica* sp.nov. Fig. 17. Dorsal view of worm. Fig. 18. Ventral view of haptor. Fig. 19. Elements of haptor, Fig. 20. Copulatory complex. Fig. 21. Vagina.

In fresh mounts the ventral bar looks like an inverted V, but in permanent mounts it tends to straighten up. It is a bar composed of two pieces, with the point of the articulation marked by a well-formed, rounded hole in the middle. The ventral bar is 0·09–0·096 mm. long. The seven pairs of hooks are all similar in shape, but differ in size. Each hook is sickle-shaped and consists of an elongated, swollen base, a very small shaft, a sickle-shaped portion and an apposable piece. The dimensions of the hooks are: pair 1, 0·029–0·032 mm.; pair 2, 0·024–0·025 mm.; pair 3, 0·006–0·007 mm.; pair 4, 0·006–0·007 mm.; pair 5, 0·006–0·007 mm.; pair 6, 0·023–0·03 mm., pair 7, 0·029–0·03 mm.

There is a conspicuous central onchium in the haptor, situated anteriorly. It is straight and elongated in shape, with rounded and slightly swollen extremities. It is 0·033–0·035 mm. long.

The testis (Fig. 17) is median, elongated, intercaecal and measures 0·065–0·071 × 0·006–0·012 mm. The vesicula seminalis was not clear in the specimens studied. The copulatory complex (Fig. 20) consists of an elongated cirrus and a similar accessory piece, both firmly articulated together at the base. The cirrus and the accessory piece are about the same length, being 0·03–0·032 mm. The cirrhal thread originates from the base and is a long thin thread. The prostate glands are not clear. The ovary is small, median and pretesticular, measuring 0·013–0·016 mm. in maximum dimensions. The vagina (Fig. 21) is dextral, with a short, funnel-shaped mouth and a transverse vaginal tube. The receptaculum seminis is not clear. No egg was observed in the specimens studied. The vitellaria are densely spread from the pharynx to the posterior end.

Discussion. The present form is assigned to the genus *Sprostonia* gen.nov., owing to the characteristic two-piece ventral bar, the articulation of the cirrus and the accessory piece at the base, and the dextral vagina. It differs conspicuously from *S. indica* gen.nov., sp.nov., and *S. wallagoni* sp.nov. by the presence of an onchium in the haptor, and from *S. gomtia* sp.nov. and *S. indica* sp.nov., by the absence of projections on the ventral anchor shafts.

It further differs from *S. indica* in (1) the shape of the cirrus, (2) the shape of the accessory piece, (3) the shape of the dorsal bar anchor bases, and (4) the shape of the hooks.

From *S. wallagoni* sp.nov. the present form differs in (1) the shape of the dorsal bar, (2) the shape of the dorsal anchor base, (3) the shape of the ventral bar, and (4) the shape of the accessory piece.

From *S. gomtia* sp.nov. the present form can be distinguished by (1) the shape of the ventral bar, (2) the shape of the dorsal and ventral anchor bases, and (3) the shape of the hook.

It is evident, therefore, that the present form is different from the species of the genus *Sprostonia* gen.nov., described earlier, and it is therefore assigned to the new species, as *Sprostonia asiatica* sp.nov.

VII. GENERIC DIAGNOSIS

Genus **Sprostonia** gen.nov.

Tetraonchinae, with several pairs of head organs and two pairs of eye-spots. Intestine bifurcate and the crura confluent posteriorly. Two pairs of anchors, dorsal and ventral. Two bars, dorsal and ventral, non-articulate in the middle. Ventral bar characteristically a two-piece bar. Hooks, 14. Onchium present or absent. Gonads in the middle of the body. Copulatory complex composed of cirrus and accessory piece, both firmly articulated at the base. Vagina dextral. Single egg. Parasites of fresh-water fishes.

Type species: *S. indica* sp.nov.

VIII. KEY TO THE SPECIES OF THE GENUS *SPROSTONIA* GEN.NOV.

1. Presence of central onchium in the haptor. 2
— Absence of central onchium in the haptor. 3
2. Conspicuous projections on ventral anchor shafts, rounded dorsal anchor bases, hooks crochet-shaped, ventral anchors with poorly developed roots
S. gomtia sp.nov.
— No projections on ventral anchor shafts, dorsal anchors with a conical knob at the base, hooks sickle-shaped, ventral anchors with well-developed roots
S. asiatica sp.nov.
3. Conspicuous projections on ventral anchor shafts, cirrus stout cork-screw like, accessory piece simple, dorsal bar with a conspicuous aperture in the middle
S. indica gen.nov., sp.nov.
— No projections on ventral anchor shafts, cirrus simple elongated, accessory piece Y-shaped, dorsal bar without an aperture in the middle. **S. wallagonia** sp.nov.

IX. SUMMARY

A new genus, *Sprostonia*, is erected in the Family Dactylogyridae, subfamily Tetraonchinae, from the study of trematodes of some Indian freshwater fishes, from Lucknow, India. The new genus differs from allied genera by the characteristically two-piece shape of the ventral bar, the articulation of the cirrus and accessory piece together at the base and the presence of a dextral vagina. The genotype *S. indica* sp.nov. was recovered from the gills of *Eutropiichthys vacha*. Three other new species, *S. wallagonia*, *S. gomtia* and *S. asiatica*, were also recovered from other Indian fishes and assigned to the new genus. The text is illustrated with twenty-one camera lucida figures and is followed by a key to the species of the new genus, *Sprostonia*.

The present work was carried out in the Department of Zoology, Lucknow University, Lucknow, under the guidance of Professor M. B. Lal. The author wishes to express his sincere thanks to him for the keen interest in the work. Acknowledgements to the Government of India are due for the award of a research training scholarship to carry out the present work and to the Lucknow University authorities for all facilities and permission to publish the work as part of the author's Ph.D. thesis, approved in 1955.

Abbreviations used in the figures

a.e.	anterior eye-spots	*ov.*	ovary
a.p.	accessory piece	*p.e.*	posterior eye-spots
c.c.	copulatory complex	*ped.*	peduncle
cir.	cirrus	*ph.*	pharynx
c.l.	cephalic lobe	*pr.*	prostate gland
c.t.	cirrhal thread	*pr.* 1 & *pr.* 2.	prostate glands no. 1 & 2
d.a.	dorsal anchor	*r.g.*	reproductive glands
d.b.	dorsal bar	*r.s.*	receptaculum seminis
d.p.	dorsal projection	*s.b.*	supporting bars
e.	egg	*t.*	testis
h. 1–*h.* 7	hooks numbered 1–7	*v.a.*	ventral anchor
h.o.	head organs	*vag.*	vagina
hp.	haptor	*v.b.*	ventral bar
h.s.	horse-shoe shaped support of vagina	*v.d.*	vas deferens
		vit.	vitellaria
int.	intestine	*vit.d.*	vitelline duct
m.g.p.	male genital pore	*v.p.*	ventral projection
m.v.	mouth of vagina	*v.t.*	vaginal tube
on.	onchium	*v.s.*	vesicula seminalis.

REFERENCES

Baugh, S. C. (1957). On the morphology of *Neogyrodactylus indicus* n.g., n.sp., a viviparous monogenetic trematode (family Gyrodactylidae) from *Argulus indicus* Weber. *Parasitology*, **47**, 40–5.

Chauhan, B. S. & Ramakrishna, G. (1953). Fauna of Belangir District (formerly Patna State), Orrisa. Part I. Fishes. *Rec. Indian Mus.* **51**, 395–426.

Hargis, W. J. jr. (1952). Monogenetic trematodes of Westhampton Lake fishes. I. Two new forms. *Amer. Midl. Nat.* **47**, 471–7.

Hargis, W. J. jr. (1953). Chloretone as a trematode relaxer, and its use in mass collecting techniques. *J. Parasit.* **39**, 224–5.

Jain, S. L. (1952*a*). Monogenea of Indian fresh-water fishes. I. *Haplocleidus gomtius* n.sp. (subfamily: Tetraonchinae) from the gills of *Wallagonia attu* (Bloch), from Lucknow. *Indian J. Helminth.* **4**, 37–42.

Jain, S. L. (1952*b*). Monogenea of Indian fresh-water fishes. II. *Thaparocleidus wallagonius* n.g., n.sp. (subfamily: Tetraonchinae), from the gills of *Wallagonia attu* (Bloch), from Lucknow. *Indian J. Helminth.* **4**, 43–8.

Jain, S. L. (1955). Monogenea of Indian fresh-water fishes. III. *Urocleidus notopterus* n.sp. (subfamily: Tetraonchinae), from the gills of *Notopterus notopterus* (Pallas), from Lucknow. *Proc. Indian Acad. Sci.* **41**, 31–7.

Jain, S. L. (1957*a*). *Mizelleus indicus* n.g., n.sp. (subfamily: Tetraonchinae), from the gill filaments of *Wallagonia attu* (Bloch). *Ann. Zool., Agra*, **2**, 57–62.

Jain, S. L. (1957*b*). Monogenea of Indian fresh-water fishes. V. *Dactylogyrus multispiralis* n.sp. (subfamily: Dactylogyrinae) from the gill filaments of *Silondia silondia* (Ham.), from Lucknow, India. *Proc. Nat. Acad. Sci. India*, **27**, B, 26–30.

Jain, S. L. (1957*c*). Monogenea of Indian fresh-water fishes. VI. Three new trematodes belonging to the genus *Neodactylogyrus* Price, 1938 (family Dactylogyridae) from some fresh-water fishes of Lucknow, India. *Proc. Nat. Acad. Sci. India*, **27**, B, 53–63.

Mizelle, J. D. (1936). New species of trematodes from the gills of Illinois fishes. *Amer. Midl. Nat.* **17**, 785–804.

Mizelle, J. D. (1938). Comparative studies on trematodes (Gyrodactyloidea) from the gills of North American fresh-water fishes. *Ill. Biol. Monogr.* **17**, 1–81.

Price, E. W. (1938). A new species of *Dactylogyrus*, with the proposal of a new genus. *Proc. Helminth. Soc. Wash.* **5**, 48–9.

SHAW, G. E. & SHEBBEARE, E. O. (1937). The fishes of Northern Bengal. *J. R. Asiat. Soc. Beng.* **3**, 1–137.

THAPAR, G. S. (1948). A new monogenetic trematode from the gills of an Indian fish, *Catla catla*, from Lucknow. *Indian J. Helminth.* **1**, 1–10.

Postscript

As this paper goes to press, Jain (1958) described a new genus, *Bifurcohaptor*, represented by two new species, *B. indicus* and *B. giganticus*, from Lucknow.

JAIN, S. L. (1958). Monogenea of Indian fresh-water fishes, VII. *Bifurcohaptor*, a new genus of fresh-water Tetraonchinae from the gill filaments of two fishes from Lucknow. *J. Parasit.* **44**, 388–94.

(*MS. received for publication* 11. IV. 1958.—Ed.)

THE OCCURRENCE OF *HISTOMONAS MELEAGRIDIS* IN *HETERAKIS GALLINAE*

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(With 2 Figures in the Text)

In 1920 Graybill & Smith demonstrated that histomoniasis could be induced by feeding young turkeys with the embryonated eggs of the caecal worm *Heterakis papillosa* (*gallinae*). Their suggestion that larval stages of the worm must be responsible for transmitting *Histomonas meleagridis* was confirmed by Tyzzer (1934) who, moreover, reported the identification of the protozoon in the epithelial cells of the gut of young *Heterakis gallinae*. The morphology of these parasites was illustrated by Tyzzer (1934), and it is clear that they were similar to *Histomonas meleagridis* found elsewhere. The illustration, however, gives little indication of the actual situation of the parasites within the tissue of the worm. Since the work of Tyzzer there has been a number of investigations into the life history of *H. meleagridis*, and it seems rather remarkable that there have been no further positive identifications of the protozoon within the worm or the worm egg.

Swales (1948), during the course of lengthy investigations, was quite unable to identify the parasite in freshly hatched larvae even though, as he said, the diameter of *H. meleagridis* was likely to be as much as half the breadth of a newly hatched larva. Subsequently, a few authors have reported the possible presence of *Histomonas* in *Heterakis*. Connell (1950) noted morphological abnormalities in second-stage larva of *H. gallinae* and suggested that these might indicate the presence of *Histomonas*. Desowitz (1950) examined female *Heterakis gallinae* recovered from turkeys dying of histomoniasis and in 1, out of 100 examined, found small intracellular parasites in the epithelium of the gut. Desowitz did not consider himself justified in identifying these parasites as developmental forms of *Histomonas meleagridis*.

At Weybridge, during the course of our investigations into histomoniasis in chickens and turkeys, it has been necessary to examine large numbers of caeca from birds previously infected with the embryonated eggs of *Heterakis*.

This paper records, with a photomicrograph and a camera lucida drawing, the occurrence of parasites resembling *Histomonas meleagridis* in a young larva of *Heterakis gallinae*.

MATERIALS AND METHODS

Young (11-day-old) turkey poults, reared in isolation and kept in wire-floored hovers, were infected orally each with 1000 embryonated eggs of *H. gallinae* from a uniform batch. The birds were killed on the 2nd, 3rd, 4th, 5th, 6th, 7th, 9th and

11th days following infection, the caeca removed, fixed in Carnoy's fluid, sectioned and stained with Giemsa's stain. Birds killed on the 9th and 11th days following infection were easily demonstrated to be heavily infected with *Histomonas meleagridis*, this indicating that the batch of worm eggs was, in fact, infected with the protozoon.

Fig. 1. A photomicrograph of a larva of *Heterakis gallinae* with protozoan parasites.

Examination of serial sections from the caeca showed the presence of very numerous larvae of *Heterakis* lying in close apposition to the epithelium, often in the crypts of the caecal mucosa or in eroded areas.

In one series of sections from the caecum of a bird killed on the 4th day after infection, organisms resembling *Histomonas* were seen within a *Heterakis* larva. These are illustrated in Fig. (1) (a photomicrograph of the larva lying in the caecal mucosa) and in Fig. (2) (a camera lucida drawing of the larva with contained parasites).

DESCRIPTION OF THE PARASITES

As shown in Figs. 1 and 2, the parasites measured about 5μ in diameter, having a conspicuous circular nucleus, lightly stained but with a well-defined nuclear membrane, one large densely stained chromatin mass and a number of small chromatin granules. The cytoplasm is granular, more densely stained and with a number of refractile bodies, presumably the lipoid granules mentioned by

Tyzzer (1934). From the figures it will be seen that four parasites are present. Of these no. (1), Fig. 2, appears in the photograph, Fig. 1, to be binucleate as if undergoing binary fission. Careful examination has, however, suggested that one of the apparent nuclei is an artifact.

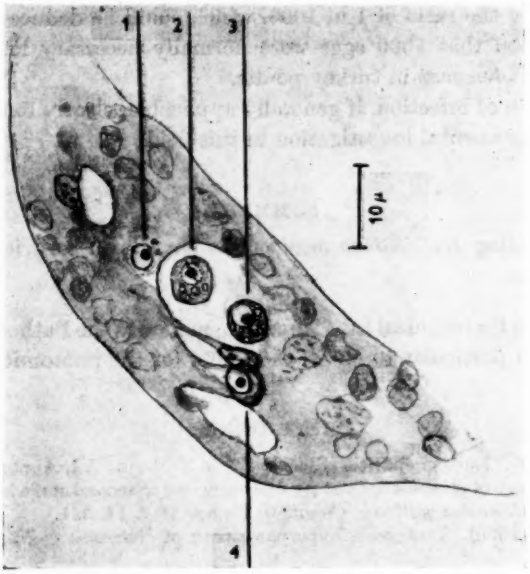

Fig. 2. A camera lucida drawing of the larva in Fig. 1 showing four parasites (1, 2, 3, 4).

The exact situation of the protozoa within the worm is difficult to assess, although from their central position it could be assumed that they are within the cells lining the gut or within the gut cavity itself. Unfortunately the characteristic elongated cells usually constituting the gut wall of nematode larvae have not been identified, and it is not certain whether the cavity containing free parasites (Fig. 2, nos. 2 and 3) is an artifact or part of the gut itself. Only a few related serial sections are available, and these unfortunately give no further indication of the identity of the tissue.

DISCUSSION

The parasites seen in the *Heterakis* larva were considerably smaller than the normal *Histomonas meleagridis* seen in tissue, but in other respects were identical. Experience at Weybridge over a number of years has shown that *H. meleagridis* can be expected to stain as did these parasites with a granular purple cytoplasm and a pale nucleus, with a well-marked nuclear membrane and irregularly distributed chromatin bodies.

It is indeed remarkable that on no other occasion have organisms which could be identified as *Histomonas* been observed in *Heterakis*. *Histomonas* is a common

parasite in Britain, nearly always transmitted under field conditions by the caecal worm, and experience at this Laboratory has indicated that all field populations of the worm are infected. The difficulty experienced in finding the protozoon in its vector may possibly be explained on the assumption that the rate of infection of the eggs of *Heterakis* (and hence of the young larvae) is very low, perhaps not greatly exceeding the ratio of 1 in 1000, which could be deduced from Kendall's (1957) observation that 1000 eggs were normally necessary in order to set up infection with *Histomonas* in turkey poults.

Such a low rate of infection, if generally applicable, clearly limits very severely the scope of experimental investigation in this field.

SUMMARY

Parasites resembling *Histomonas mealagridis* have been seen in a young larval *Heterakis gallinae*.

Thanks are due for technical assistance to members of the Pathology Department at Weybridge, in particular to Mr E. Clements, for the photomicrography.

REFERENCES

CONNELL, R. (1950). Enterohepatitis (Blackhead) in Turkeys. VI. Abnormalities, probably caused by a stage of *Histomonas meleagridis* occurring in second stage larvae of blackhead-transmitting *Heterakis gallinae*. *Canad. J. Comp. Med.* **14**, 331.

DESOWITZ, R. S. (1950). Protozoan hyperparasitism of *Heterakis gallinae*. *Nature, Lond.*, **165**, 1023.

GRAYBILL, H. W. & SMITH, T. (1920). Production of fatal Blackhead in Turkeys by feeding embryonated eggs of *Heterakis papillosa*. *J. Exp. Med.* **31**, 647.

KENDALL, S. B. (1957). Some factors influencing resistance to histomoniasis in turkeys. *Brit. Vet. J.* **133**, 435.

SWALES, W. E. (1948). Enterohepatitis (Blackhead) in Turkeys. II. Observations on transmission by the caecal worm (*Heterakis gallinae*). *Canad. J. Comp. Med.* **12**, 97.

TYZZER, E. E. (1934). Studies on histomoniasis, or 'Blackhead' infection, in the chicken and the turkey. *Proc. Amer. Acad. Arts Sci.* **69**, 189.

(*MS. received for publication* 22. IV. 1958.—Ed.)

THE MIGRATION OF *CERCARIA X* BAYLIS (STRIGEIDA) WITHIN THE FISH INTERMEDIATE HOST

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(With Plates III and IV, and 6 Figures in the Text)

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I. INTRODUCTION

Cercaria X Baylis is a longifurcous, pharyngeal furcocercaria emerging from sporocysts present in *Lymnaea stagnalis* from Roath Park Lake, Cardiff. Previous studies (Erasmus, 1958*b*) have shown that this cercaria will penetrate a wide range of vertebrate hosts and develop in the lens of the eye into a strigeid metacercaria of the diplostomulum type. The prevalent natural host is the three-spined stickleback (*Gasterosteus aculeatus*) and the continued development of the cercaria will take place only in the lens of the eye. This rigid specificity poses a number of interesting problems, particularly in relation to the migration of the cercaria from the point of entry into the fish body to its final location in the lens.

Very many observations have been made over the last century on strigeid cercariae and metacercariae and a number of attempts have been made to explain the specific location of some of the metacercarial forms. Thus Steenstrup (1845) stated that he sometimes noticed 'a finely granular, unorganized streak, which, passing through the cornea to the "pupa" (metacercaria), closely indicated the way by which the animal had penetrated previously to becoming a pupa'. Blochmann (1910), who had lost some of the fish in his aquarium as a result of cercarial penetration, showed that the migration of the cercariae in the fish caused their death. He found large numbers of cercariae in the heart, heart blood and brain and noticed numerous small haemorrhages in the pericardial region, in the region of the gill arteries and also in the orbit and lens. Szidat (1924), working with *Cercaria C*, examined fishes that had died after cercarial infection and found cercariae under the skin, in the brain, in the gill region where the main blood vessels were full of

cercariae, in the pharynx and in the orbit. He suggested that the nerve tracts and blood vessels formed easy routes along which the cercariae could travel. Dubois (1929) studied in serial sections the migration of *Cercaria letifera* in small trout. He noted that the head was most frequently attacked with the cercariae penetrating very rapidly. In heavy (fatal) infections cercariae were found in all the main blood vessels, in the heart, skin, connective tissue, mucous membrane lining the mouth, in the body muscles and in the brain. Wesenberg-Lund (1934) noticed that the external surface of fish exposed to the attacks of cercariae was not covered with creeping cercariae and suggested that most of the cercariae were drawn in through the mouth, or entered by the skin. In dead fishes he found tail-less cercariae everywhere, creeping on the gills, below the scales, in the stomach, in the heart and in the lens of the eye, great numbers being present in the veins of the gills and in those to the heart. In his view there was no doubt that the cercariae were carried with the blood stream into the body.

None of these early workers studied the problem experimentally and, in spite of the similarity in their observations, the exact route of migration was not established. These studies, however, showed (*a*) that penetration into the body may occur either through the skin, or, as first suggested by Wesenberg-Lund, through the gills; (*b*) that in fatal infestations cercariae are found everywhere in large numbers both in and out of the blood stream. Thus their suggestions that the blood stream is the path of migration are not conclusive.

An experimental approach to the study of the problem has been made by Davis (1936) and Ferguson (1943). Davis studied the penetration of *C. flexicauda* into tadpoles. Examination of dead specimens revealed a number of haemorrhages in the body and many cercariae in the brain, eye, lens, lumen of the heart, liver, gills, kidneys and intestinal tract. He concluded that migration of cercariae was via the blood stream because of the speed of their localization. Infection of the tadpoles was accomplished by placing their tails in water containing cercariae and, as he was able to recover cercariae from the eye within three hours, he assumed that this rapid migration must have occurred via the blood stream. This evidence is again rather circumstantial.

Ferguson (1943) studied the problem in more detail using the same cercaria but the more natural host—the fish. By an elaborate series of surgical experiments he was able to show that cercariae did not localize in orbits from which the eye had been removed. In addition, ligature of the optic blood vessels and cutting of the optic nerve prevented the localization of cercariae in otherwise intact eyes. From these and other results he deduced that the blood stream was the route of infection and that entry into the eyeball was via the optic blood vessel. Thus these experiments tended to provide information on the factors affecting localization rather than on details of the actual migration.

The following problems still seem to be outstanding. In a normal, non-fatal infection: (1) Does entry into the body occur irregularly all over the surface, or does it occur more frequently in one region? (2) Is the lens reached by a definite route or by chance migration? (3) Do the cercariae migrate through the blood stream or through the tissues, or both? The experiments described in this paper

represent an attempt to obtain more detailed information on these aspects of migration. In addition, some observations have been made on penetration and on the effects of the migrating cercariae on the fish.

II. METHODS

All experimental infections were carried out with newly emerged *Cercaria X* from *Lymnaea stagnalis*. Cercariae were obtained in the following manner. Infected *L. stagnalis* were isolated overnight in 3 × 1 in. tubes. After a reasonable number of cercariae had emerged the suspenion was decanted into a graduated cylinder. A small sample (0·2 ml.) was removed and the number of cercariae counted. This sampling was repeated six times. From the samples the approximate number of cercariae in the suspension was obtained and the suspension was then diluted to give the volume and cercarial concentration required for infection. The fish used in the experiments were small (2·5–4·0 cm. long) three-spined sticklebacks. They were fixed by dropping the entire fish into hot 10% formalin. Death was instantaneous and after about 1 hr. in the fixative the fish was cut up into three or four pieces. This was not done immediately after death, as cercariae might have been lost with blood escaping after cutting. Fish were decalcified in Gooding and Stewart's formic acid mixture (Carleton & Leach, 1938), washed, dehydrated and double embedded following Peterfi's (Carleton & Leach, 1938) procedure.

For the study of the relative numbers migrating in the different tissues it was assumed that the host tissue or tissues consistently containing the greatest number of cercariae would be the tissue most frequently entered and traversed by the migrating cercariae. If, for example, the blood stream consistently contained the highest number of cercariae during the first 24 hr. after infection, it would be assumed that the cercariae migrated most frequently through the blood stream. It was also assumed that the migration of cercariae from one tissue to another would be indicated by appropriate changes in the numbers from different tissues.

In practice the following procedures have been adopted. Each fish was serially sectioned at 10μ, and the sections mounted on 2 × 3 in. slides. Every sixth section (i.e. nos. 1, 7, 13, 19, etc.) in the series was carefully studied and a record made of the number of cercariae in each tissue type. The average length of a cercarial body in this fixed material was 105μ and would extend over a consecutive series of approximately ten sections. Thus, by examining every sixth section, very few cercariae were missed as the majority of the cercarial bodies were extended parallel to the longitudinal axis of the fish. To simplify the handling of the numbers and to aid interpretation, the numerical information has been telescoped into consecutive units of 550μ long and composed of ten examined sections. Because of the length of the cercarial body the same cercaria would occur in two consecutively examined sections and would be counted twice. To reduce this error the total number of cercariae in a single tissue in each unit was halved. This number is referred to as the Tissue Number. The total count covering all the tissues in the ten sections in one unit is described as the Unit Total and the Fish Total represents the total count throughout the whole fish. The other figure used in the interpretation of the results is the Tissue Percentage which is the sum of the tissue numbers

of a particular tissue throughout the entire fish expressed as a percentage of the fish total.

The component tissues of the fish body were grouped into categories, each of which was given a code letter. These code letters were used in the construction of tables and a key is given below:

A.C.	Alimentary canal	*Liv.*	Liver
B. cav.	Body cavity	*Lns.*	Lens
B.S.	Blood system	*M.*	Muscle
C.T.	Connective tissue; fatty tissue	*N.S.*	Nervous system
E.S.	External surface	*Sk.*	Skin and mucous membrane of pharynx
Gl.	Gills—filaments; rakers, etc.	*Sk. Sy.*	Skeletal system
Go.	Gonads and ducts	*Sp.*	Spleen
Hm.	Humours	*W.*	Wall of alimentary canal
Kd.	Kidney and ducts	*W.L.*	Wall—conjunctival, choroid, sclerotic and retinal layer
L.	Lumen of alimentary canal		

In text-figs 1–5 the approximate extent of (1) the eye is measured from the anterior to the posterior point of the orbit and (2) the head, measured from the tip of the snout to the posterior edge of the pericardium.

All sections were stained with Weigert's haematoxylin with Ponceau S as counterstain.

Photographs of living material were taken with electronic flash synchronized to a single-lens, 35 mm., reflex camera attached to the microscope (Erasmus, 1958*a*).

Observations on penetration were carried out on living and pithed fish. In addition to the stickleback, isolated tail fins of male *Lebistes reticulatus* were mounted under a coverslip in cercarial suspension to which a few drops of saturated aqueous solution of Alizarin Red S had been added. The stain coloured the secretions as they emerged from the openings of the penetration gland cell ducts. Purpurin used in the manner described by Stirewalt & Hackey (1956) did not stain either the gland or the secretions. With Alizarin Red S the gland cells were coloured a pale amber, but the secretions as they emerged took on a bright orange colour. Tail fins of the Guppy (*L. reticulatus*) were particularly suitable for these preparations because of their length and the presence of numerous melanophores, which allowed certain observations, described later, to be made on the actual migration.

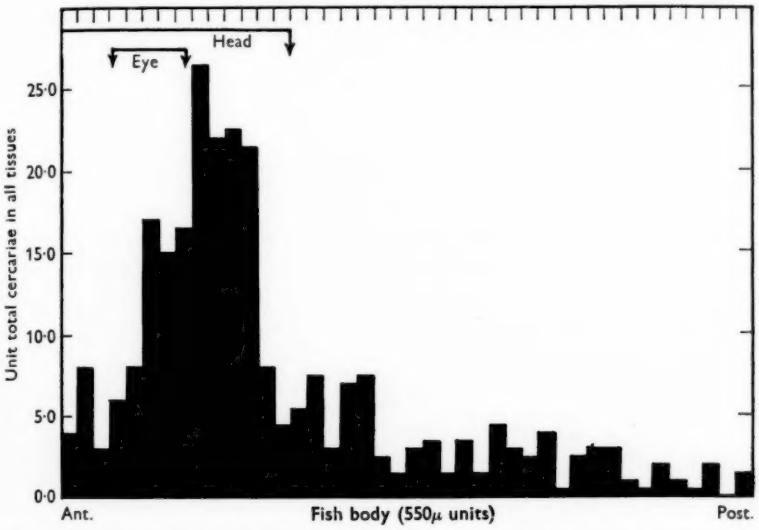
III. PENETRATION INTO THE FISH BODY

(*a*) *Site of penetration*

In an attempt to gain information on the main site or sites of entry of the cercariae into the fish the following experiment was performed. A small stickleback was exposed to the attack of approx. 9000 newly emerged *Cercaria X* in 30 ml. of water for 3 min. After this period the fish was removed to clean water and 57 min. after exposure to infection fixed in 10% formalin. The distribution of cercariae shown in Text-fig. 1 indicates that cercariae were able to enter along the whole length of the fish, although a marked concentration occurred in the head region, particularly in the region of the gills. The figures used in the construction

of Text-fig. 1 represent cercariae migrating deep within the tissues as well as those in the skin. A more detailed analysis of this cercarial distribution is given in Table 1, which contains the tissue numbers in the various tissues throughout the entire body. These figures show that entry into the fish is not confined to the external surface, but that a number of cercariae are drawn into the pharynx on the respiratory current and are filtered out by the gills.

This suggestion is supported by two other observations. First, the characteristic anchor-shaped floating position (see Pl. III, fig. 1) of the cercariae is the ideal



Text-fig. 1. Overall distribution of migrating *Cercaria X* in *Gasterosteus aculeatus* 57 min. after infection. Cercarial concentration 9000 in 30 ml.; fish exposed for 3 min.

Table 1. *Distribution of* Cercaria X *in* G. aculeatus 57 *min. after infection*

	Tissue numbers/region					
Region of body ...	*Sk.*	*C.T.*	*M.*	*B.S.*	*Sk. Sy.*	Unit total region
Tip of snout to ant. face of eye (3 units)	3·5	9·9	1·5	0·0	0·5	15·0*
Eye (4 units)	18·0	21·0	3·5	0·5	2·5	46·0†
Post. face of eye to end of head (7 units). Region of gills	31·0	34·0	5·5	20·0	2·5	121·5‡
Trunk and tail (28 units)	39·5	25·0	4·0	7·5	2·5	79·0§
Sum of tissue numbers	92·0	89·0	14·5	28·0	8·0	261·5 fish total

Unit totals include: * *E.S.* = 0·5; † *W.L.* = 0·5; ‡ *Gl.* = 27·5; § wall of *A.C.* = 1·0. *B. cav.*; *L.*; *Liv.*; *N.S.*, *Sp.*, *Go.*, *Kd.*, *Lns.*, *Hm.* = 0·0.

shape to be caught by the gills. Secondly, if the gills are removed from a fish which has been exposed to cercariae and examined under a microscope it may be seen that numbers of tail-less cercariae are present both on the gill surface and actually penetrating into the gills. The absence of the tail is an important feature as its loss under such conditions is an indication of attempted tissue penetration by the cercarial body. The table also indicates that this anterior concentration is due to a combination of the following factors: (1) cercariae drawn along with the respiratory current first come in contact with the head region and penetrate the skin on the head: (2) penetration also occurs via the gills; (3) because of this double source (gills and skin) of cercariae the numbers in the connective tissue are higher relative to the rest of the body, (4) as a consequence of gill penetration more cercariae are present in the anterior blood system than in the system posterior to the heart.

(*b*) *Method of penetration*

The actual penetration of the cercaria into the fish body has been observed on isolated fish tails, pithed fish and living fish. The cercaria, after coming into contact with the host tissue, attaches itself by the ventral sucker. The firmness of this attachment is suggested by Pl. III, fig. 4. Here the cercaria has released the hold of the ventral sucker upon the tissue, leaving a plug of tissue in the shape of an internal mould of the ventral sucker and representing the previous point of attachment. In addition to this muscular adhesion the ventral sucker bears two alternating rows of hooks, which provide further anchorage to the tissue. After attachment the cercaria extends and contracts the portion of the body anterior to the ventral sucker, 'testing' the substratum with the oral sucker. Movement across the host surface is leech-like, consisting of alternate attachment by oral and ventral suckers, coupled with extension and retraction of the body. The tail plays no part in this movement and generally hangs still except for an occasional flicker from side to side. By the addition of Alizarin Red S it has been possible to stain the secretions of the penetration gland cells. This secretion may be expelled in the form of droplets from the cercaria in the complete absence of host stimulus. In aqueous media the secretion is very viscous and sticky and in temporary aqueous preparations cercariae occasionally get stuck to the coverslip by the droplets. As the oral sucker presses against the host surface, the droplets are extruded and it seems probable that the secretion has an adhesive function in this initial contact with the host. Eventually penetration occurs by the thrusting of the oral sucker into the host tissue (Pl. III, figs. 5, 6). The passage through the tissues is mainly accomplished by the activity of the oral sucker (anterior organ) and the apical cap of spines. A regular retraction and eversion of the apical cap takes place, thrusting and splaying the large apical hooks into the tissue and at the same time the penetration gland cells release their secretions. This movement of the apical cap is very regular and the sequence everted–inverted–everted takes about 10 sec. when the cercaria is migrating through connective tissue. The tail does not seem to play a great role in penetration. Usually it lies still and all the activity is confined to the body region anterior to the ventral sucker. The tail is generally cast off

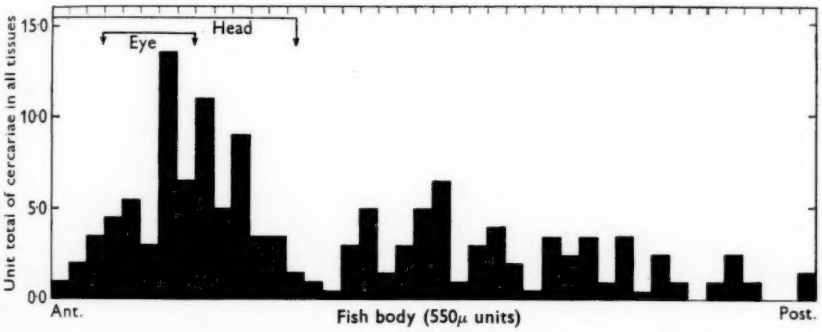
before the body completely enters the host (Pl. III, figs. 2, 3), or occasionally it is drawn into and plugs the mouth of the entry tunnel. Penetration is rapid and is accomplished in about 7–8 min.

IV. MIGRATION

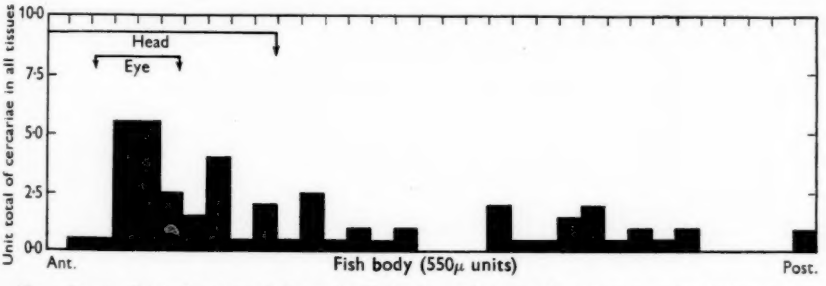
(*a*) *Gross movement*

Text-fig. 1 represents the distribution of cercariae 57 min. after infection. Subsequent alterations of this distribution were investigated by the following experiments.

Experiment 12. Two fish exposed to approx. 3900 *Cercaria X* in 100 ml. of water for 5 min were removed to fresh water and fixed, 55 min. (A) and 5 hr. 37 min. (B) after infection.



Text-fig. 2. Distribution of *Cercaria X* in *G. aculeatus* 55 min. after infection. Cercarial concentration 3900 in 100 ml.; fish exposed for 5 min.

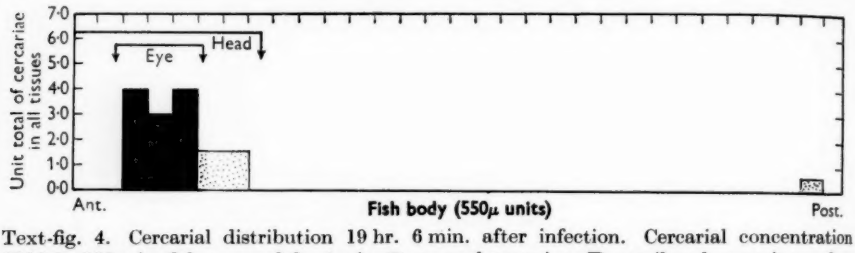


Text-fig. 3. Distribution of *Cercaria X* in *G. aculeatus* 5 hr. 37 min. after infection. Cercarial concentration 3900 in 100 ml.; fish exposed for 4 min.

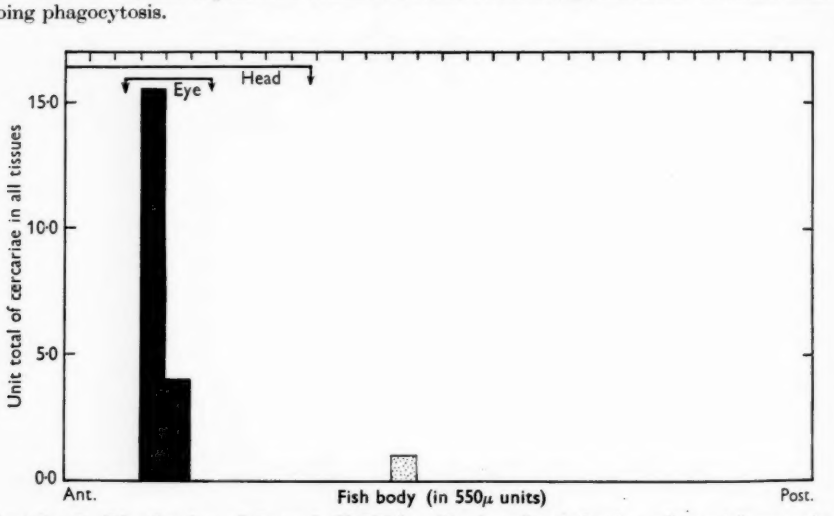
Experiment 15. Two fish exposed to approx. 3300 *Cercaria X* in 100 ml. for 4 min. were removed to fresh water and fixed 19 hr. 6 min. and 139 hr. 49 min. after infection. The overall distribution of cercariae in these four fish is illustrated in text-figs 2–5.

The distribution after 55 min. (Text-fig. 2) shows the same general pattern as that shown in Text-fig. 1, although the latter represents an infection with a much

larger number of cercariae. Cercariae have entered and are present along the entire length of the body, but again there is a peak in the head, particularly in the region of the gills. For the next few hours (5 hr. 37 min.,Text-fig. 3) the overall distribution has not altered and cercariae are still present within the entire length of the fish. This general distribution does not persist, and after 19 hr. 6 min. (Text-fig. 4) has altered markedly. It has become much more limited and is concentrated almost entirely within the head region, where, however, distribution in the component tissues is still quite general. In the rest of the body there is only an isolated specimen in the tip of the tail. This localization becomes much more pronounced as



Text-fig. 4. Cercarial distribution 19 hr. 6 min. after infection. Cercarial concentration 3300 in 100 ml.; fish exposed for 4 min. ■, normal cercariae; ▤, moribund cercariae undergoing phagocytosis.



Text-fig. 5. Distribution of *Cercaria X* 139 hr. 49 min. after infection. Cercarial concentration 3300 in 100 ml.; fish exposed for 4 min. ■, normal cercariae; ▤, moribund cercariae undergoing phagocytosis.

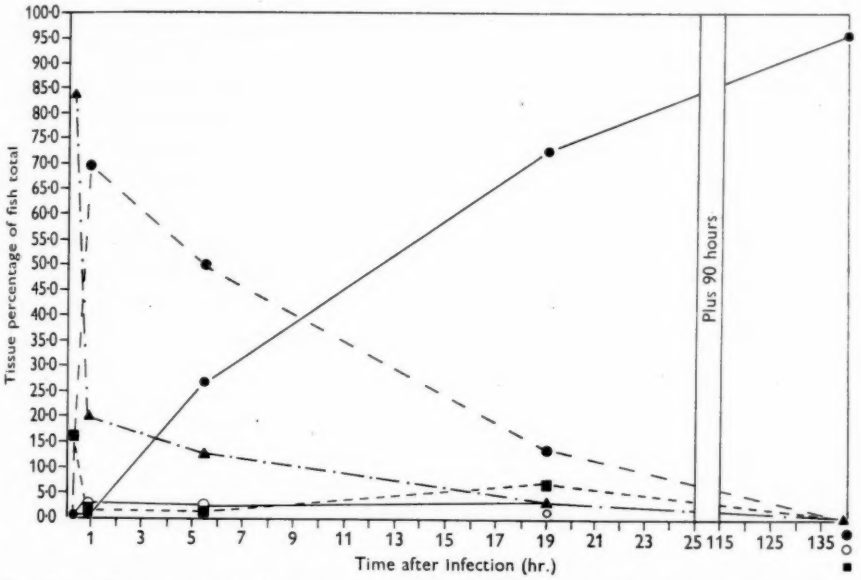
the time after infection increases (Text-fig. 5, 139 hr. 49 min.). After this period localization is most rigid, all cercariae being confined, not only to the head region but more strictly to the lens itself. This distribution represents the rigid tissue specificity exhibited by the metacercarial stage (Erasmus, 1958*b*).

Immediately after infection, cercariae are distributed throughout the entire body but, as the time after infection increases, so the distribution becomes more

and more restricted, until localization is confined to the specific host tissue parasitized by the metacercaria. This localization occurs rapidly and is completed within 24 hr. after infection.

(*b*) *Distribution in various tissues*

Details of the percentage distribution of cercariae within all the major tissue groups in relation to the time after infection are given in Table 2. A graph based on some of these figures and illustrating the cercarial movement between certain tissues is given in Text-fig. 6. Fifteen minutes after infection all the cercariae were concentrated in the skin and gills. Fifty-five minutes after infection the



Text-fig. 6. The variation in the percentage of cercariae migrating in various tissues in relation to time after infection. ●——●, Eye; ●- - -●, connective tissue + muscle; ○——○, blood system; ▲-·-·-▲, skin; ■- - -■, gills.

Table 2. *Relative distribution of* Cercaria X *in various tissues in relation to time after infection*

Time after infection	Tissue percentage of fish total							Eye	
	Sk.	*C.T.*	*M.*	*Gl.*	*B.S.*	*N.S.*	*Sk. Sy.*	*W.L.*	*Lns.*
15 min.	84·2	0·0	0·0	15·8	0·0	0·0	0·0	0·0	0·0
55 min.	19·5	59·0	10·6	1·8	3·1	0·75	4·5	0·0	0·0
5 hr. 37 min.	12·8	20·7	29·7	1·4	2·8	2·8	1·4	15·5	5·1
19 hr. 6 min.	3·4	3·4	10·3	6·9	3·4	0·0	0·0	0·0	72·6
139 hr. 49 min.	0·0	0·0	0·0	0·0	0·0	0·0	4·8	2·5	92·7

W. at 55 min. only = 0·75; *E.S.*, *Liv.*, *Sp.*, *Go.*, *Kd.* = 0·0; *B. cav.* at 5 hr. 37 min. only = 1·4; *Hm.* at 5 hr. 37 min. only = 6·4.

majority (69·6 %) of the cercariae were present in the connective tissue and muscle layers and only a small percentage (3·1 %) occurred in the blood stream.

Within the first 55 min. after infection the proportion in the connective tissue and muscle rises very rapidly. Then, as time after infection increases, so the proportion of cercariae present in these tissues falls rapidly from 69·6 to 13·7 % within 19 hr. 6 min. after infection. In a similar manner there is an initial concentration of cercariae in the skin representing those that have penetrated, and this rapidly falls off as the cercariae migrate deeper into the body tissues. The numbers in the eye show an inverse relationship to those present in the muscle and connective tissue. Immediately after infection there are very few cercariae in the eye, but the numbers increase rapidly until, as shown in Text-fig. 6, 95·2 % of the cercariae in the body are restricted to the eye. The proportion of cercariae in the blood vessels remains low, never rising above 3·5 %.

These figures suggest that, although penetration and entry may occur all over the fish, this non-localized entry does not result in a haphazard distribution of cercariae within the body tissues. The abdominal cavity with its contained organs does not seem to be traversed by the migrating cercariae. Considering the large numbers of cercariae which must enter the pharynx in the respiratory stream, the almost complete absence of cercariae from the alimentary canal is very surprising. The majority of the cercarial movements seem to take place within the connective tissues and muscle layers, with a small, relatively constant proportion occurring in the blood stream.

(*c*) *The rate and route of migration*

Localization in the eye occurs fairly rapidly. Five and a half hours after infection 27·0 % are present in the eye and this increases to 72·6 % within 19 hr. after infection. Thus it seems reasonable to assume that localization is almost completed within the first 24 hr. after infection, and considering the distances some of the cercariae have to migrate, this represents a very good rate of passage through the tissues. Using an experimental preparation of tail fin, an approximate estimation of the rate of migration has been made. The cercariae were migrating in the connective tissues between the fin rays and traversed a distance of 0·4 mm. in about 8 min. This rate will vary with the type of tissue being traversed, and entry into and passage along a major blood vessel would result in a more rapid migration through the fish. Taking these factors into consideration, it seems probable that the cercariae reaching the eye within 5 hr. are those which entered the fish body in the head region via the skin and gills.

The actual route of migration received much consideration as has already been mentioned in the introduction. The majority of authors favour the blood stream as the main route, basing their conclusions chiefly on the rate of migration. However, Table 2 shows that only a relatively small percentage of the cercariae migrating in the body are present in the blood system. Thus, taking the entire body into consideration, the evidence presented above does not support the hypothesis that the blood stream forms the main route of migration. The variation in the numbers present in the blood stream along the length of the body is shown in

Table 1. These results indicate that the majority of cercariae migrating within the blood stream occur in the head region in the vicinity of the gills. This numerical indication is strongly supported by direct examination of the slides. In the region anterior to the heart, cercariae have been frequently observed within blood vessels, particularly the afferent vessels (see Pl. III, fig. 7) and the ventral aorta, and it seems very probable that cercariae entering the fish via the gills frequently penetrate these major blood vessels in this region and use them for migration. This would account for the relatively high proportion of cercariae present in the vessels of the head. The suggestion that the blood stream, although it does not contain large numbers of cercariae at a given moment, may receive a low but steady number of cercariae from the connective tissues, and may in this way form a major route to the eye, is not supported by Text-fig. 6. Here the rate of disappearance from the connective tissue and muscle is very rapid and a corresponding rate of increase in numbers occurs only in the eye. If the blood stream forms an important link between connective tissue and the eye, it seems remarkable that the numbers in it are so consistently low. This feature could be explained by an extremely rapid passage along the blood vessel, once it has been entered. However, the size and activity of the cercariae are too great to allow it to be swept passively along with the current, and therefore the rate of progress will be governed by the activity of the cercariae rather than by the rate of blood flow. Observations tend to suggest that such extremely rapid rates of progress are not generally attained. In addition, as mentioned earlier, the majority of cercariae migrating in the blood stream occur in the head and these can be accounted for by the cercariae penetrating the gills and pharynx.

Table 3. *Relative distribution of* Cercaria X *in various tissues of orally infected fish*

Time after infection	Tissue percentage of fish total							Eye	
	Sk.	*C.T.*	*M.*	*Gl.*	*B.S.*	*N.S.*	*Sk. Sy.*	*W.L.*	*Hm.*
40 min.	2·2	56·6	0·0	32·7	6·4	0·0	0·0	2·1	0·0
4 hr. 30 min.	1·1	29·8	1·1	10·7	15·9	7·5	1·0	9·5	23·4

E.S., A.C., Liv., Sp., Go., Kd., Lns. = 0·0.

This hypothesis was further tested by the following experiment. A number of fish were orally infected with *Cercaria X*. Newly emerged cercariae were concentrated by centrifugation into a small volume (3–5 ml.) of water. The fish was removed from the water, held in the left hand and the concentrated cercarial suspension was then introduced slowly with a pipette held in the right hand. In this way large numbers of cercariae were introduced into the pharynx. The fish was returned to the water, allowed to recover for a few minutes and then removed again. This time the outer surface of the fish was cleaned by holding the fish in running water and brushing the external surface with a camel hair brush. The fish was finally returned to water. Table 3 gives the proportion of cercariae in the

various tissues of fish infected in this manner 40 min and 4½ hr. after infection. Because of the almost complete elimination of the cercariae which normally entered through the skin and were migrating in the trunk, it is possible to see more clearly the role played by the blood stream during migration from the gills. Comparing these figures with those from normally infected fish and at similar times after infection, it is apparent that the cercariae migrating in the blood stream of normally infected fish may be almost entirely accounted for by those which have penetrated the gills and have then moved into the afferent vessels and ventral aorta. In addition, the experiments not only indicated that cercariae could reach the eye from the gills, but also confirmed the suggestion that cercariae present in the eye within 5 hr. have migrated from the gills and pharynx. It is important to emphasize that even in these experiments the proportion migrating in the blood stream is very small compared to the numbers present in the connective tissue and muscle.

Within the rest of the body tissues the number of migrating cercariae is very small. The body cavity is rarely traversed and the organs in it seem never to be entered; and in a chance migration this is very difficult to explain. In the case of the nervous system it is possible that the protective nature of the skeletal covering would account for the low number of carcariae recorded from this tissue. However, this does not explain their marked absence from other organs. Further evidence against the concept of chance migration may be derived from Text-figs. 2–5. If the migration was non-directional, it would be reasonable to assume that the original cercarial concentration in the head would be dissipated throughout the entire body. The evidence indicates the contrary. Not only does this concentration persist, but it becomes even more restricted as the time after infection increases. There is then no longer any general distribution throughout the entire body.

This disappearance of cercariae from the post-orbital regions of the body may be the result of a number of factors. It may imply that all the cercariae migrate forwards and enter the eye. The alternative explanation is that the majority of cercariae migrate forwards, but that a small number, for a variety of reasons, fail to complete the migration and become immobilized and are finally phagocytized by the host cells. If the latter interpretation is correct, it means that the host tissues can react actively within 24 hr., as at 19 hr. after infection a large number of cercariae have already disappeared from the post-orbital regions of the body. Careful examination of the sections of a fish killed 19 hr. 6 min. after infection shows that all the cercariae present in the head region outside the eye are moribund and appear to be undergoing phagocytosis (Pl. IV, fig. 8). The work of Smith (1931), on wounding in gold fish, suggested that cell movements begin relatively early and he noted the presence of melanophores, which appear some time after the tissue reaction has been initiated, at the wound sites 2 days (at 25·6° C.) after the initial tissue damage. In contrast to this, Hunter & Hamilton (1941), describing the migration of cercariae and their development into the encysted metacercariae of *Uvulifer ambloplitis*, stated that 48 hr. after infection there was no evidence of host tissue reaction and that they could not find macrophages until the 5th–7th

day. The observations here recorded support Smith's (1931) conclusions that host cell reactions begin early, but there will obviously be great variation depending on the temperature of the external environment, the severity of the initial stimulus and nature and condition of the host animal.

The migration within the blood vessels seems to be relatively temporary, as cercariae may be frequently observed entering and leaving the blood vessels. Entry into the eye is not restricted to any particular route and penetration into the eyeball may occur at any point, even through the external surface of the conjunctiva. After passage through the wall of the eye, the cercariae traverse the humours and enter the lens (Pl. III, figs. 4, 5).

V. EFFECTS OF MIGRATION ON THE FISH

When a fish is exposed to the attack of cercariae its behaviour becomes abnormal; within a few minutes it becomes restless and dashes around the tank. This spasmodic activity alternates with periods of calm. Small fish exposed to large numbers of cercariae die very rapidly. Small minnows an inch long succumbed after 3 min.

The combined action of the spines and the secretions of the penetration gland cells during migration has been described above, but the function of the gland cell secretions in relation to migration is rather obscure. As mentioned earlier, the secretion when it is extruded into an aqueous medium is viscous and sticky. Observations on cercariae migrating within Alizarin Red S preparation of an isolated fish tail showed that the secretion does not remain concentrated in front of the cercarial body, but, because of the alternate expansion and contraction of the anterior part of the body, tends to spread out backwards over the cercarial body. The secretion remains in contact with the tissue in front of the body only for a few seconds, so that any digestion which occurs would have to take place very rapidly. The highly pigmented tail fin of male *Lebistes reticulatus* made it possible to observe what was happening immediately in front of the cercaria. If one assumed that a lytic secretion was expelled anterior to the cercarial body, it seemed probable that the melanophores lying in the migratory path just ahead of the cercaria would exhibit some disturbance, but it was always noted that the melanophore wall was not ruptured until the oral spines came into contact with it. Occasionally a cercaria would penetrate the tail fin completely in a transverse direction and the rim of the resultant aperture was always stained orange-red by the Alizarin Red S coloured secretion (Pl. IV, fig. 2). These observations on *Cercaria X* confirm certain statements made by Stirewalt & Hackey (1956) in which they also commented on the viscous and sticky nature of the secretions from the pre-acetabular gland cells of cercariae of *Schistosomum mansoni* penetrating mammalian skin. They also noticed that the droplets tended to remain as discrete entities within the migration tunnels and also stained the circumference of the apertures of the penetration tunnels. Thus it seems possible that the secretions of the gland cells of *Cercaria X* are not lytic but may be adhesive and/or lubricatory in function. This concept is partly supported by the histochemical studies of Lewert & Lee

(1954), who were unable to detect any collagenase-like activity in extracts of an unidentified strigeid cercaria. A lubricatory function has also been suggested for the secretions of certain Xiphidiocercariae (Kruidenier, 1951, 1953).

Whatever the mechanism, there is no doubt that the migration of cercariae within the fish body causes extensive damage. The exact extent of this damage is unpredictable as the route which the cercariae follow cannot be defined exactly. Penetration into the skin results in the disruption of the epidermis at various points (Pl. III, figs. 4–6) and occasionally results in interference with the lateral line sense organs. In the post-cardiac region of the body, migration occurs mainly in the muscle layers, and the previous passage of cercariae is often indicated by the track of damage left by the cercariae (Pl. IV, fig. 6). Presumably damage of this nature is of secondary importance and would not be fatal. Migration within the blood stream may produce more severe results. In its most innocuous form the presence of carcariae in the smaller vessels may result in a temporary blockage. More severe and often fatal damage is caused by the migration of cercariae into and out from the vessels (Pl. IV, fig. 1). As mentioned earlier, migration within the circulatory system occurs most frequently in the vessels anterior to the heart. In this way the ventral aorta often becomes severely damaged by the perforation and migration of cercariae in the muscle coat (Pl. IV, fig. 7). In a fish exposed to 9000 *Cercaria X* in 30 ml. for 4 min. and fixed $5\frac{1}{2}$ hr. after infection, eight complete perforations were noted in 1·2 mm. of ventral aorta anterior to the heart, together with two cercariae migrating in the wall. In such cases a great deal of internal bleeding would occur and this could easily be the cause of death. Cercariae are occasionally found in the heart, and here again similar factors, perforation and haemorrhage, blockage and destruction of muscle layers, would soon lead to the death of the host.

In a number of instances cercariae have been found migrating in the brain with resultant destruction of nervous tissue. This latter condition, coupled with interference with the lateral line sense organs and destruction of retinal tissue (Pl. IV, fig. 3) during entry into the eye, may explain the orientation defects which sometimes occur in fish after infection. In such cases the fish has to exert itself to maintain orientation in the water and in a severe condition, unless a continuous movement of the fins were maintained, the fish floated with the abdomen uppermost. Generally, if the fish survived a period of 24 hr. after infection, its behaviour would return to normal. The temporary nature of this disturbance supports the concept that the majority of cercariae have localized in the lens within 24 hr. and that the remainder are already being rendered innocuous by the host tissues.

VI. DISCUSSION

The experiments described above indicate that cercarial penetration may occur at any point on the body of the fish coupled with a local concentration in the gill region. The overall distribution resulting from such an infection is at first general, but within 24 hr. after infection it becomes more and more restricted, until it is confined to the eye and ultimately to the lens itself. Cercariae present outside the

eye after this period appear to be moribund and in most cases are undergoing phagocytosis. The majority of migrating cercariae are present in connective tissue or muscle. Only a small proportion (2–4 %) migrate in the blood system and of these most occur in the blood vessels in the immediate vicinity of the gills. Cercariae rarely appear in the brain or body cavity with its contained organs.

These results confirm and explain some of the observations made by Blochmann (1910), Szidat (1924), Dubois (1929) and Wesenburg-Lund (1934), but they do not support the hypothesis put forward by these and later workers that the blood stream forms the main route for migration. There is no doubt that the cercariae enter the blood stream and traverse it for short periods, particularly in the region of the gills, but, of all the cercariae that reach the lens, only a small proportion have migrated in this manner. The rapid rates of migration recorded by Davis (1936) and Ferguson (1943) may be explained by the fact that no apparent attempt was made to prevent cercariae creeping over the surface of the host and entering in the immediate vicinity of the eye. Ferguson's (1943) suggestion that entry into the eye was by way of the optic blood vessel is not supported by observations recorded here on this particular combination of host and parasite and the evidence presented above shows that there is no localized point of entry into the eye. Early workers have supplied some confirmatory evidence. Blochmann (1910) commented on the large numbers of cercariae penetrating the tissues of the eye, and also described much tissue damage in the retina caused by migrations of the cercariae. Szidat (1924) observed cercariae in the sclerotic below the external pigment layer, in the capillary zone of the choroid and in the rods and cones of the retina. Ferguson's conclusion was based mainly on the fact that, when the optic blood vessels were cut or ligated, no cercariae entered the eye. He also found that cutting of the optic nerve reduced the number of cercariae entering the eyeball. As these experiments were not supported by histological evidence of cercariae within these blood vessels, it is suggested that as a result of these surgical procedures, the normal functioning of the eye was so altered that the cercariae did not or could not localize in it.

Whether or not the migration is a directive one still remains to be proven, but a number of observations may be added to those already published. First, the very uniform experimental results of Ferguson (1943) strongly suggest that the physiological state of the eye influences to a marked extent the success of the migration. A more detailed appraisal of this conclusion is not possible at present. It may be that the cercariae are capable of reaching the eye but migrate out of it again as a result of its abnormal physiological state. Secondly, the figures illustrating the movement of the cercariae within the body of the fish after infection tend to support the concept that the migration is directive. The distribution is essentially unimodal and this, instead of dispersing, becomes more strongly emphasized.

Not all the cercariae reach the eye. It seems that if localization is not completed within 24 hr. the cercariae become moribund. Previous studies (Erasmus, 1958*b*) have indicated that the cercarial body contains extensive glycogen reserves. It is possible that the duration of the migration may be limited by the amount of glycogen present. As the reserves become depleted, so the cercarial body becomes

less active. The condition of the penetration gland cells may also play a part. Ferguson (1943) showed that when cercariae, which had completed their migration to the eye and in which the gland cells were largely used up, were injected into the orbit they were unable to complete their migration. However, in view of the uncertainty regarding the nature of the secretions of the gland cells, it is difficult to assess accurately the role of these structures in migration.

VII. SUMMARY

1. Studies, using serial sections and a method of numerical assessment of the numbers of cercariae in the various tissues, have shown that cercarial entry occurs through the skin at any point along the length of the fish and also through the gills and pharynx in the head region. The distribution of penetration along the length of the body is not uniform but exhibits a marked concentration in the head region.

2. As the time after infection increases, this initial unimodal distribution is maintained and becomes even more restricted.

3. Nearly all the cercariae have localized in the eye 12–24 hr. after infection. The few that remain outside the eye after this period appear to be moribund and undergoing phagocytosis.

4. The majority of migrating cercariae are present in connective tissue and muscle. Very few occur in the blood system and even fewer in the other organs of the body. The numbers in the blood system show a local anterior concentration in the blood vessels anterior to the heart and in the region of the gills. It seems probable that the blood system does not form the major route of migration.

5. Entry into the eye may occur at any point.

6. Cercarial migration causes extensive tissue damage, but this is fatal only when major blood vessels are perforated and severe internal haemorrhage results. Interference with the nervous system and lateral line system may be the cause of the orientation disturbances which are sometimes noted within 12 hr. after infection.

7. It is suggested that migration through the tissues is achieved mainly by the action of the anterior spination and that the function of the penetration gland cells may be lubricatory and/or adhesive rather than lytic.

8. The restricted tissue distribution during migration and the retention of an essentially unimodal distribution of cercariae along the length of the body throughout the whole period of migration suggest that localization in the lens is not achieved by chance migration.

REFERENCES

BLOCHMANN, F. (1910). Sterben von Aquarienfischen durch Einwanderung von *Cercaria fissicauda* La Val. *Zbl. Bakt.* **56**, 47–9.

CARLETON, H. M. & LEACH, E. H. (1938). *Histological Technique*, 2nd ed. London: O.U.P.

DAVIS, D. J. (1936). Pathological studies on the penetration of the cercariae of the strigeid trematode *Diplostomum flexicaudum*. *J. Parasit.* **22**, 329–38.

DUBOIS, G. (1929). Les cercaires de la region de Neuchâtel. *Bull. Soc. neuchâtel. Sci. nat.* **53**, 3–177.

ERASMUS, D. A. (1958*a*). Notes on the use of electronic flash in photomicrography. *Nature, Lond.*, **181**, 1290.

ERASMUS, D. A. (1958*b*). Studies on the morphology, biology and development of a strigeid cercaria (*Cercaria X* Baylis, 1930). *Parasitology*, **48**, 312–35.

FERGUSON, M. S. (1943). Migration and localization of an animal parasite within the host. *J. Exp. Zool.* **93**, 375–403.

HUNTER, G. W. & HAMILTON, J. M. (1941). Studies on host-parasite reactions to larval parasites. IV. The cyst of *Uvulifer amploblitis*. *Trans. Amer. Micr. Soc.* **60**, 498–507.

KRUIDENIER, F. J. (1951). The formation and function of mucoids in virgulate cercariae including a study of the virgulate organ. *Amer. Midl. Nat.* **46**, 660–86.

KRUIDENIER, F. J. (1953). Studies on the function of mucoids in cercariae: non-virgulate Xiphidiocercariae. *Amer. Midl. Nat.* **50**, 382–97.

LEWERT, R. M. & LEE, C. L. (1954). Studies on the passage of helminth larvae through host tissues. I. Histochemical studies on the extracellular changes caused by penetrating larvae. II. Enzymatic activity of larvae *in vivo* and *in vitro*. *J. Infect. Dis.* **95**, 13–51.

SMITH, G. M. (1931). The occurrence of melanophores in certain experimental wounds of the goldfish *Carassius auratus*. *Biol. Bull.* **61**, 73–84.

STEENSTRUP, J. J. (1845). On the alternation of generations. Translated from the German version of C. H. Lorenzen by George Busk. *Ray Soc. Monog.*

STIREWALT, M. A. & HACKEY, J. (1956). Penetration of host skin by cercariae of *Schistosoma mansoni*. I. Observed entry into skin of mouse, hamster, rat, monkey and man. *J. Parasit.* **42**, 565–80.

SZIDAT, L. (1924). Beiträge zur Entwickelungsgeschichte der Holostomiden. II. *Zool. Anz.* **61**, 249–66.

WESENBERG-LUND, C. (1934). Contributions to the development of the Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters. *K. danske vidensk. Selsk.* (Sec. Sci. 9), Ser 5, 1–223.

EXPLANATION OF PLATES

PLATE III

Fig. 1. Characteristic anchor-shaped floating posture of *Cercaria X*. Living material. Electronic flash. (× 90.)

Fig. 2. Tail fin of male *Lebistes reticulatus* with attached cercarial body and cast off tails. Living material. Electronic flash. (× 70.)

Fig. 3. *Cercaria X* penetrating tail fin of male *L. reticulatus*. Cercarial body has entered tissues and forked tail still attached is held erect, away from the fin. Living material. Electronic flash. (× 80).

Fig. 4. Section showing *Cercaria X* penetrating skin of *Gasterosteus aculeatus*. Note heavily stained tissue plug at previous point of attachment of ventral sucker. Ehrlich's haematoxylin and eosin. (× 370.)

Fig. 5. *Cercaria X* migrating below epidermis of *G. aculeatus*. Note tunnel produced by cercarial body and disruption of host epidermis at head end. Ehrlich's haematoxylin and eosin. (× 370).

Fig. 6. Migration to subcutaneous tissue. Note body shape which reflects the active body movements which occur within the tissues. Penetration gland cells still evident. Weigert's haematoxylin and Ponceau S. (× 370.)

Fig. 7. T.S. afferent vessel of *G. aculeatus* containing cercarial bodies. Ehrlich's haematoxylin and eosin. (× 460.)

PLATE IV

Fig. 1. *Cercaria X* penetrating an artery in the head of *Gasterosteus aculeatus*. Ehrlich's haematoxylin and eosin. (× 340.)

Fig. 2. Hole through tail fin of male *L. reticulatus* produced by migration of *Cercaria X*. The rim of the aperture is deeply stained by the Alizarin Red S present in the preparation. Living material. Electronic flash. (× 90.)

Fig. 3. *Cercaria X* passing through retinal tissues. Note disruption of pigment layer. Ehrlich's haematoxylin and eosin. (× 340.)

Fig. 4. Cercaria in process of entering lens of *G. aculeatus*. Ehrlich's haematoxylin and eosin. (× 340.)

Fig. 5. Cercarial body lying beneath lens epithelium of *G. aculeatus* after completion of migration. Ehrlich's haematoxylin and eosin. (× 340.)

Fig. 6. Portion of track left by migrating cercarial body in muscle blocks of *G. aculeatus*. Note complete destruction of muscle fibres. Weigert's haematoxylin and Pinceau S. (× 360.)

Fig. 7. T.S. ventral aorta of *G. aculeatus* containing body of migrating cercaria. Note also perforation of aorta wall diagonally opposite cercarial body. Weigert's haematoxylin and Ponceau S. (× 150.)

Fig. 8. Cercarial body which has failed to complete its localization undergoing phagocytosis in muscle block of *G. aculeatus* approximately 19 hr. after infection. Weigert's haematoxylin and Ponceau S. (× 360.)

(*MS. received for publication* 28. IV. 1958.—Ed.)

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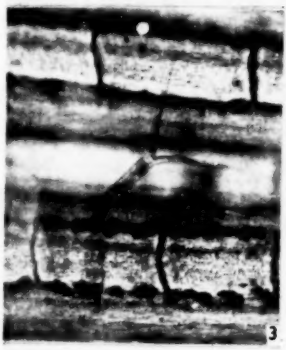

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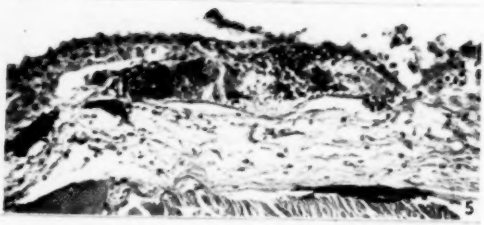

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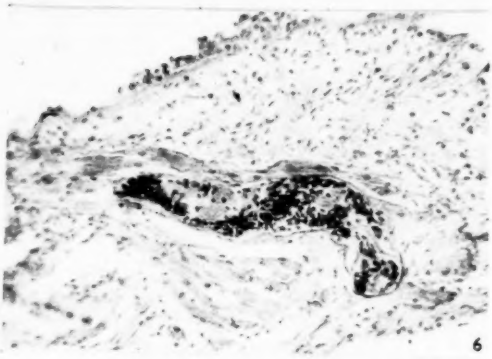

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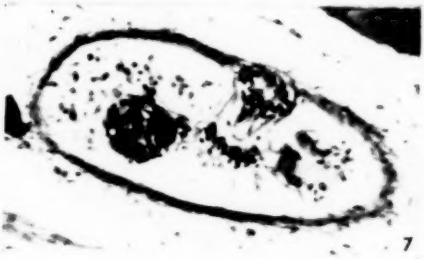

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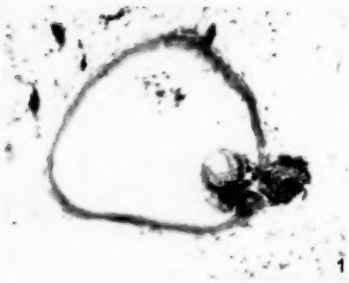
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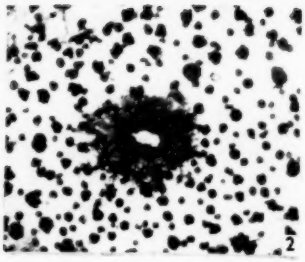
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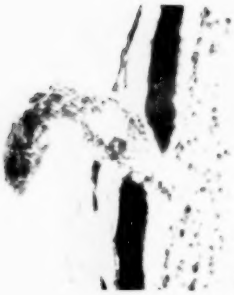
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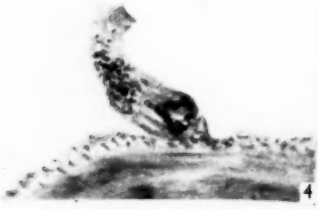
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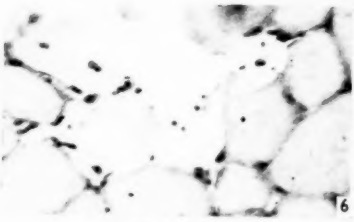
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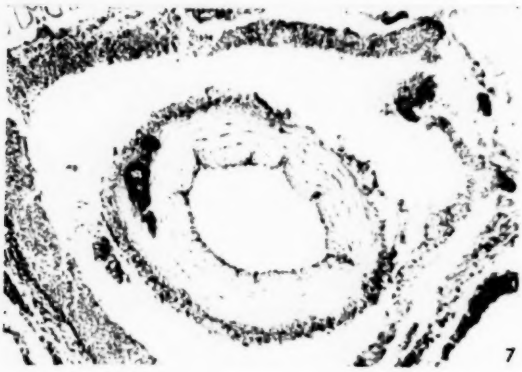
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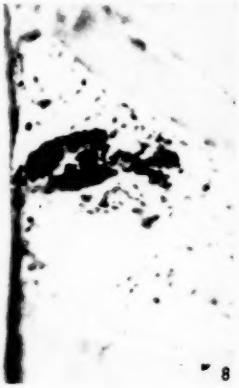
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DITRACHYBOTHRIDIUM MACROCEPHALUM GEN.NOV., SP.NOV., A CESTODE FROM SOME ELASMOBRANCH FISHES

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(With Plate V and 27 figures in the Text)

Specimens of *Ditrachybothridium macrocephalum* gen.nov., sp.nov. were obtained from the spiral valve of three species of elasmobranch fishes, *Raja fullonica* L., *R. circularis* Couch and *Scyliorhinus caniculus* (L.), caught in the trawl in September 1957 in the fishing grounds off the west coast of Scotland near the island of St Kilda. I am greatly indebted to Dr H. H. Williams and Mr D. Hemingway Jones, B.Sc., of this department who collected the material. Details of the occurrence of the worm and the localities from which it was obtained are as follows:

Host	Date	Locality	Depth (fathoms)	No. of specimens
Raja fullonica	16. ix. 57	57° 50′ N., 9° 15′ W.	160	Two small
R. fullonica	16. ix. 57	57° 50′ N., 9° 0′ W.	130	Two small
R. fullonica	17. ix. 57	57° 50′ N., 9° 15′ W.	160	Two large, two small (two fragments)
R. circularis	17. ix. 57	57° 50′ N., 9° 0′ W.	130	Two small, (one fragment)
Scyliorhinus caniculus	17. ix. 57	57° 50′ N., 9° 15′ W.	160	Two small

The specimens were collected during five separate trawling operations on two consecutive days in almost the same locality. They were fixed at the time of collecting in 5% formaldehyde. Whole mounts were stained with Gower's Carmine or Celestin Blue and serial sections cut at $7\frac{1}{2}\,\mu$ in thickness were stained with Heidenhain's azan or Ehrlich's haematoxylin and Orange G. Spiral valves of several specimens of *Raja fullonica* were also fixed in 5% formaldehyde and brought back to the laboratory for examination. Portions of the fold of the wall of one of these were sectioned and stained with Heidenhain's azan or Tiroxy-haematin.

The worms, with the exception of the few fragments, were complete, the last segment being present in each case. The small specimens were immature, some showing no signs of reproductive organs and others, rudiments only of the genitalia. In the two large specimens recovered from *R. fullonica* on 17 September the last

segment showed the reproductive system in the mature condition in one (Pl. V, fig. 5) and very nearly so in the other (Pl. V, figs. 1, 4). No gravid segments were found.

The largest specimen measured 56·063 mm. in length with a maximum breadth of 0·500 mm. in the posterior fourth of the body. It consisted of a scolex and fifty segments. The other large specimen measured 31·50 mm. in length with a maximum breadth of 0·425 mm. and consisted of a scolex and fifty-nine segments. The length of the small complete worms varied from 10·02 to 16·00 mm. with a diameter of 0·341–0·573 mm. according to the degree of contraction. These small specimens tended to curl up in a flat dorso-ventral spiral of about one and a half turns if dropped into fixative without flattening.

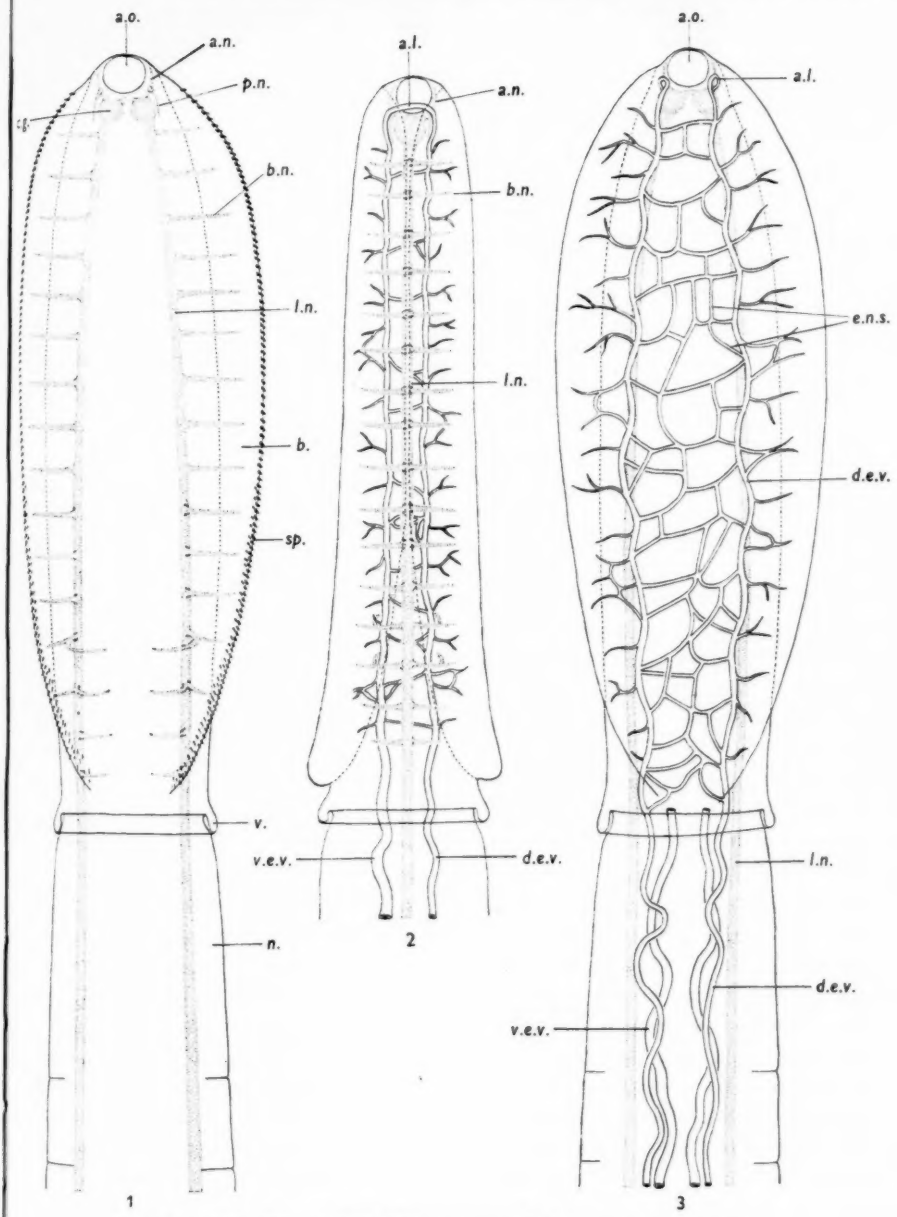
Ditrachybothridium macrocephalum is white in colour, elongated and tapers slightly towards the anterior extremity. The segmentation is not clearly visible to the naked eye and the strobila is cylindrical in unflattened specimens.

Scolex

The scolex is elongated, measuring 1·30–1·43 mm. in length in the largest flattened specimens (Text-figs. 1–3; Pl. V, fig. 2). In the smallest, which was unflattened and in which the scolex was contracted, it measured 0·872 mm. in length. The scolex bears a dorsal and a ventral bothridium, each of which is oval or spoon-shaped in outline, sessile and occupies almost the whole length of the scolex. The bothridia are broadest, usually, at about one-third of their length from the anterior extremity where in flattened specimens they measure 0·414 mm. in breadth. In contracted specimens the breadth is greatest at about the middle region (Pl. V, fig. 3) and may reach 0·573 mm. The bothridia narrow fairly rapidly to a bluntly pointed anterior extremity and more gradually towards the posterior extremity. At both ends they pass insensibly into the scolex. The exposed surface of each bothridium, i.e. the dorsal or ventral face respectively, is flat or slightly concave (Text-figs. 1–3, 11–18). The bothridia are broader than the scolex itself and so project beyond it laterally for most of their length. Posteriorly, where the bothridia narrow, the scolex becomes apparent on either side (Text-figs. 1, 3 and 18, Pl. V, fig. 2). The outer adjacent surfaces of the dorsal and ventral bothridia, where they extend laterally, are convex, the wall curving from its point of attachment to the scolex to meet the flattened exposed surface (Text-figs. 12–18). The transverse diameter and the depth of the scolex increase slightly from the anterior to the posterior ends, as will be seen from the following series of five measurements, in millimetres, taken at fairly equal intervals along its length:

	Anterior to posterior end				
Scolex, diameter	0·134	0·224	0·319	0·341	0·346
Bothridia, diameter	0·202	0·370	0·400	0·317	0·273
Scolex plus bothridia, depth	0·109	0·134	0·141	0·142	0·146

It can be seen that there is a steady increase in the diameter of the scolex and an increase followed by a decrease in the diameter of the bothridia. There is also a slight gradual increase in the depth of the scolex in the same series. As a result of this



Text-figs. 1–3. *Ditrachybothridium macrocephalum* gen.nov., sp.nov.

Text-fig. 1. Scolex, dorsal or ventral view, showing bothridium with marginal spines and the nervous system.

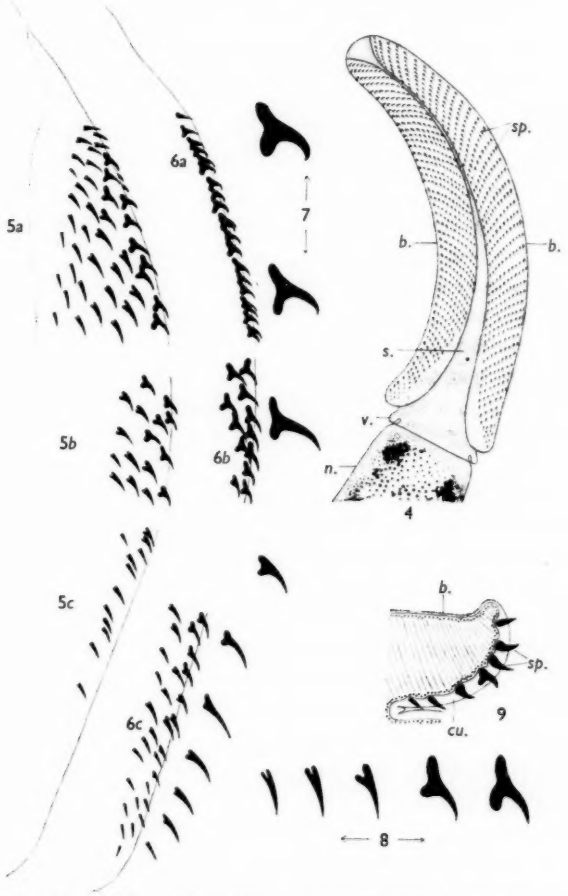
Text-fig. 2. Scolex, lateral view, showing nervous and excretory systems.

Text-fig. 3. Scolex, dorsal view, showing dorsal half of excretory system.

and of the narrowing of the bothridia the latter gradually diverge from one another laterally in the posterior half of the scolex (Text-figs. 2, 4 and 18). In unflattened specimens, and presumably in the living condition, the exposed face of each bothridium sometimes becomes more concave so that the outer or lateral adjacent surfaces are rolled away from one another towards the dorsal and ventral surfaces on either side (Text-fig. 4). Behind the bothridium is what might be described as a short cephalic peduncle, terminated by a velum which marks the posterior limit of the scolex (Text-figs. 1–4). The velum measures 0·312–0·350 mm. in breadth and 0·036 mm. in depth. The anterior extremity of the scolex is bluntly pointed and a transverse section through this region shows a spherical area or apical organ 0·085 mm. in diameter demarcated by a layer of delicate muscle fibres (Text-figs. 1–3, 10–11). Within this area there is apparently no development of gland cells or any special arrangement of muscle fibres. This feebly developed apical organ may help to maintain some degree of rigidity at the tip of the scolex.

The bothridia are armed with spines (Text-figs. 1, 2, 4, 5–9 and 12–18; Pl. V, fig. 2). In a surface view of the bothridia the spines can be seen along the right and left lateral margins commencing a short distance behind the anterior end and continuing almost to the posterior limit (Text-fig. 1). In the anterior half there appear to be two alternating rows on either side (Text-fig. 6*a*, *b*) but more posteriorly, as the bothridia narrow, the spines encroach slightly on to the surface until there are three or four rows on either side (Text-figs. 1, 6*c*). The true arrangement of the spines is best seen in a lateral view of the scolex or in transverse sections where they are found to cover the outer convex surface of each bothridium on either side (Text-figs. 4, 9 and 12–18). Those spines on the margins of the bothridia, in surface view, are in effect the first of a series of alternating rows which extend from the margin, obliquely, along the convex outer surface to the point of junction of the bothridia with the lateral wall of the scolex. A somewhat similar arrangement of spines has been described by Baylis (1950) for the four bothridia of *Dinobothrium spinosum*. Anteriorly and posteriorly there is a slight decrease in the number of spines per row (Text-figs. 4, 5). The spines have a characteristic shape, those along the anterior half of the margins of the bothridia are stout and thorn-shaped with backwardly directed hook-like tips (Text-fig. 6*a*). They measure 0·0146 mm. in length. Passing backwards they become slightly less robust (Text-fig. 6*b*) until finally they are small straight spines measuring only 0·007 mm. in length (Text-fig. 6*c*). Text-fig. 7 illustrates a series of spines from along the margin of the bothridium, showing the change in size and shape from the anterior to the posterior ends. A similar change in the shape of the spines is apparent on the outer convex surface of each bothridium (Text-fig. 5*a–c*). Passing from the margin along the outer convex surface, they become gradually more slender, the root and point less well defined, until eventually in the depth of the lateral groove they are reduced to simple straight spines (Text-figs. 5*a*, 8 and 9). The arrangement of the spines and the variation in their shape is presumably correlated with their function. Those at the posterior ends of the bothridia and those in the depth of the lateral grooves are least likely to come in contact with the surface to which the scolex must attach itself and so are reduced in size, while those in the middle region and along most of

the free margins are the best developed. As already stated, in unflattened specimens the exposed dorsal and ventral faces of the bothridia become concave, causing the edges to roll over, exposing more of the spiny convex outer surfaces (Text-fig. 4).



Text-figs. 4–9. *Ditrachybothridium macrocephalum* gen.nov., sp.nov.

Text-fig. 4. Scolex, lateral view, showing outer convex face of the dorsal and ventral bothridia rolled over, exposing the rows of backwardly directed spines.

Text-fig. 5 (*a*), (*b*) and (*c*). Arrangement of spines on the anterior, middle and posterior regions of the outer convex face of the bothridium, surface view.

Text-fig. 6 (*a*), (*b*) and (*c*). Arrangement of spines on the anterior, middle and posterior regions of the margin of the bothridium, surface view.

Text-fig. 7. Series of ten spines taken at intervals from the anterior to the posterior ends of the margin of the bothridium, showing progressive decrease in size.

Text-fig. 8. Series of five spines selected from those of one row on the outer convex surface of the bothridium, showing progressive decrease in size from the margin inwards.

Text-fig. 9. Transverse section through the free margin of one bothridium, showing arrangement of spines on the outer convex surface.

If such a head is thrust between the villi of the wall of the spiral valve of the host, the spines could be embedded in neighbouring villi, anchoring the worm in position. Pl. V, fig. 6, is a vertical section through a fold of the spiral valve of *Raja fullonica* and shows the narrow spaces between the villi into which the scolex of *Ditrachybothridium macrocephalum* could be thrust. Unfortunately, no specimens were found still adhering to the wall of the intestine. Possibly the attachment is not as firm as in some other species with more powerfully developed scolices, so the worm loses its grip on fixation.

The spines are embedded in the cuticle, which is much thicker in the regions where they occur than on the rest of the scolex (Text-figs. 9, 12–18). They occupy the whole depth of the cuticle and appear to encroach slightly into the underlying parenchyma. They are, however, limited internally by the basement membrane and no muscles have been seen attached to their roots. The spines, therefore, are presumably incapable of independent movement, their position depending on the degree of contraction and expansion of the scolex and bothridia and on the rolling of the margins of the latter.

There is no armature at the apex of the scolex nor on the short cephalic peduncle. The latter terminates in a velum which overlaps, slightly, the proliferating zone which follows.

Strobila

The scolex is followed by a neck or proliferating zone (Text-figs. 1, 3) which in the longest specimen measured 1·91 mm. in length and 0·202 mm. in breadth. The strobila is acraspedote. The early segments, which are not very clearly marked, are about ten times broader than long. Later they become square and finally about four or five times longer than broad (Text-fig. 23; Pl. V, figs 1, 5). The first segment in this same worm measured 0·288 mm. broad and 0·027 mm. long. A segment from about a quarter of the way down the strobila measured 0·400 mm. broad and 1·320 mm. long and one at half-way 0·425 mm. broad and 2·000 mm. long. The maximum length and breadth of the segments occur at about three-quarters of the length along the complete strobila where a segment measured 0·500 mm. in breadth and 2·446 mm. in length. The last segment in the complete worm is usually a little smaller than those immediately preceding it. In this case it measured 0·414 mm. in breadth and 1·872 mm. in length. In the second largest specimen (Pl. V, figs, 1, 4) and in one of the smaller ones the last segment was for some unknown reason much broader than the remainder of the strobila. Rudiments of the genitalia appear early and gradually develop towards the posterior end. As already stated, with the exception of the last segment of the longest worm (Text-fig. 23) no fully mature segment was found. The genital atrium is in the mid-ventral line in the posterior quarter of the segment, the cirrus sac being in front of the vagina. There is no uterine pore. The strobila is cylindrical in unflattened specimens (Text-fig. 22).

The cuticle on the strobila and on the exposed unarmed faces of the bothridia consists of two layers, an outer composed of closely set 'hairs' and an inner homogeneous layer (Text-figs. 11–20, 25–27). It is thinnest on the dorsal and ventral faces of the bothridia where it measures 0·002 mm. In the early part of

the strobila it measures 0·007 mm. and more posteriorly 0·009 mm. in thickness. On the outer convex surfaces of the bothridia, which bear the spines, the cuticle is 0·009 mm. in thickness, more than four times as thick as that on the flattened surfaces. Here it consists entirely of a homogeneous layer, devoid of 'hairs', in which the spines are embedded. There are no 'hairs' on the apical or lateral regions of the scolex or on the cephalic peduncle (Text-figs. 10, 18 and 19). In the latter the cuticle measures 0·009 mm. in thickness.

Musculature

The musculature of *Ditrachybothridium macrocephalum* is not well developed.

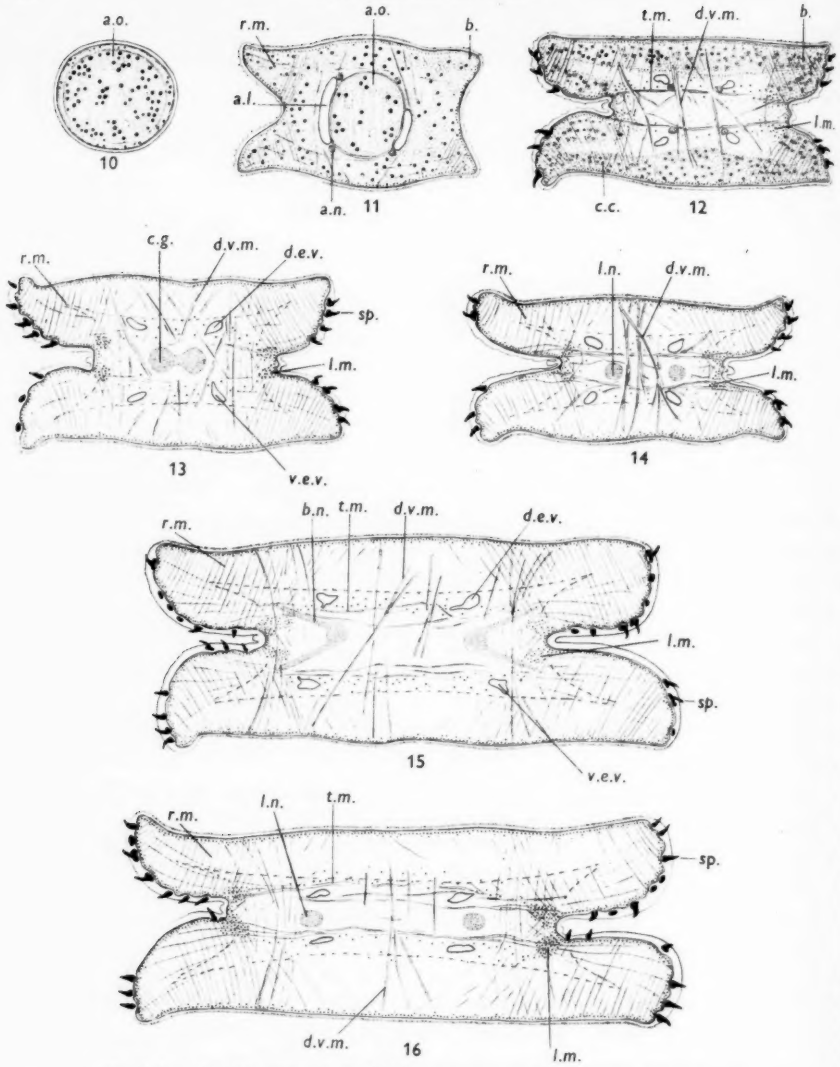
(*a*) *Superficial musculature* The superficial musculature throughout the scolex and strobila consists of one or two layers each of circular and longitudinal muscle fibres (Text-figs. 10–20, 25–27).

(*b*) *Deep musculature*

Scolex. The bothridia are simple structures not sharply demarcated from the underlying parenchyma. They are not very contractile and the musculature is somewhat unspecialized, being in the form of a network extending without interruption through the scolex and bothridia. Transverse, dorso-ventral, radial and longitudinal muscles contribute to the general network.

(1) *Transverse muscles.* At the anterior extremity of the scolex a few delicate transverse muscle fibres pass across from the right to the left anterior borders of the bothridia dorsally and ventrally (Text-fig. 11). They are inserted into the basement membrane on either side. Immediately behind the apical organ the transverse muscles are better developed and form a dorsal and ventral band in the central zone of the scolex to the inner side of the dorsal and ventral excretory vessels (Text-fig. 12). Some anastomosis takes place in the central zone between the two layers of fibres which are inserted into the basement membrane, laterally, in the depth of the groove between the dorsal and ventral bothridia. The transverse muscles continue in much the same pattern throughout the length of the bothridial region of the scolex (Text-figs. 13–18). In places they encroach to a greater or lesser degree into the central zone or occasionally to the outer sides of the excretory vessels. Some extend into the bothridia, where the latter are widest, and spread out fan-wise to be inserted into the basement membrane on the outer convex faces of the bothridia (Text-figs. 15, 16). The transverse muscles are best developed in the anterior half of the scolex (Text-figs. 12–16). Towards the posterior half they become gradually more diffuse (Text-figs. 17, 18) and where the bothridia finally become continuous with the scolex, posteriorly, they are very delicate and only a few remain in the cephalic peduncle.

(2) *Dorso-ventral muscles.* Dorso-ventral muscles are present throughout the length of the scolex. They are very delicate at the anterior end where they consist of a few fibres passing from the dorsal to the ventral body wall on either side of the apical organ (Text-fig. 11). Immediately behind the latter and in front of the brain they become prominent in the central zone where they are arranged in bundles of a few fibres each (Text-fig. 12). Anastomosis takes place between fibres of neigh-



Text-figs. 10–16 *Ditrachybothridium macrocephalum* gen.nov., sp.nov.

Text-fig. 10 Transverse section through tip of scolex, showing feebly developed apical organ.

Text-fig. 11. Transverse section through scolex, showing beginnings of bothridia and dorso-ventral excretory loop on either side of the apical organ.

Text-fig. 12. Transverse section through scolex immediately behind apical organ and in front of brain showing bothridia with spines, excretory vessels and the transverse, dorso-ventral, radial and longitudinal muscles.

Text-fig. 13. Transverse section through the scolex, showing cerebral ganglia united by transverse commissure and the musculature.

Text-fig. 14. Transverse section through the scolex immediately behind the cerebral ganglia, showing the lateral nerves and the better developed musculature in the region.

Text-fig. 15. Transverse section through the scolex, showing the bothridial nerves arising from the lateral nerves; the bothridia, musculature and excretory vessels as before.

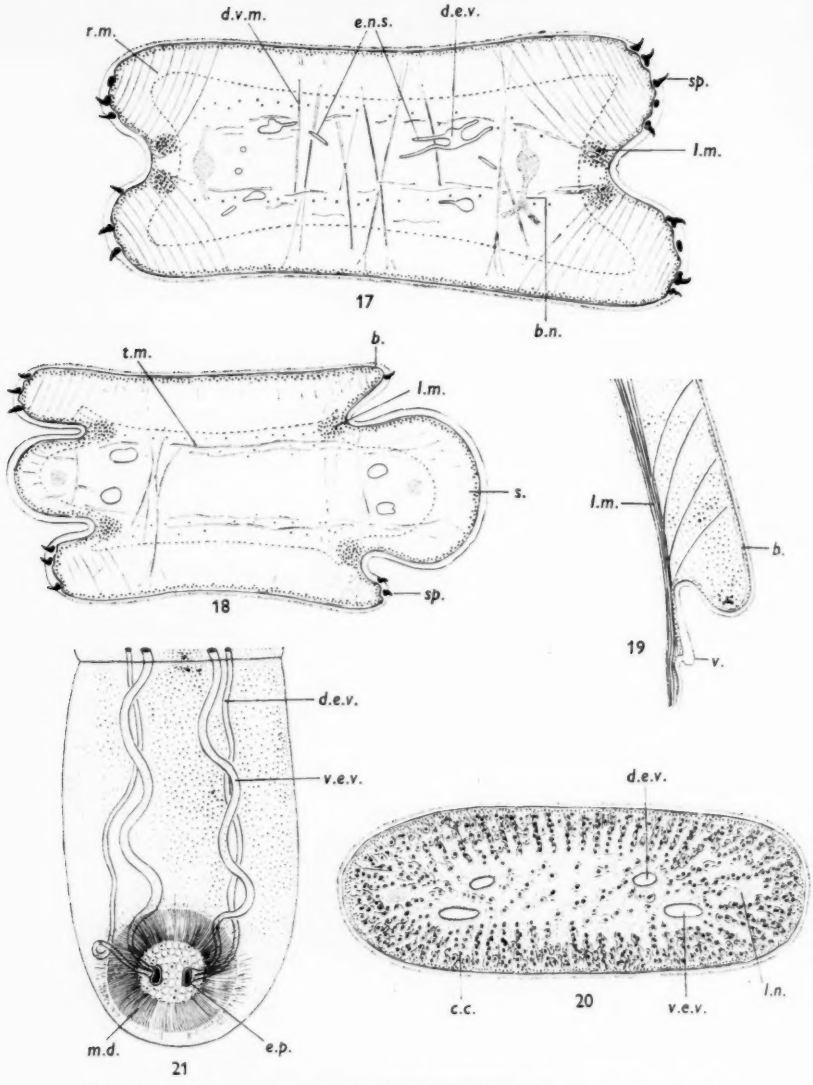
Text-fig. 16. Transverse section through the scolex, showing, mainly, extension of the radial muscles towards the centre.

bouring bundles so that some appear to pass obliquely across the central zone. The dorso-ventral muscles are interrupted by the brain, passing to either side of it or above and below it (Text-fig. 13). Behind the brain they are again well developed, forming several bundles in the central region (Text-fig. 14). The dorso-ventral muscles continue throughout the scolex (Text-figs. 15–18), extending in places laterally beyond the excretory vessels and lateral nerves. Outside the lateral nerves they tend to curve outwards dorsally and ventrally to extend into the bases of the bothridia (Text-figs. 15, 16). Approaching the posterior end of the scolex the lateral nerves diverge from one another so that the dorso-ventral muscles again tend to be confined between them. In the cephalic peduncle they are in the form of a few delicate fibres distributed across the scolex in the area between the lateral nerves.

(3) *Radial muscles.* The radial muscles are confined, anteriorly, to the lateral regions of the bothridia, but further back in the scolex they encroach into the central zone. At the anterior end they consist of a few fibres passing diagonally across the as yet inconspicuous margins of the bothridia (Text-fig. 11). As the bothridia become more prominent the muscles become more extensive. For the most part they are evenly distributed in the free lateral regions, the innermost fibres of the dorsal and ventral sides meeting in the lateral groove (Text-fig. 12). In the neighbourhood of the brain (Text-fig. 13), and in some other places in the scolex, they may extend farther in so that some of the radial fibres from the dorsal and ventral sides cross one another opposite the lateral grooves and to the outer sides of the lateral nerves (Text-figs. 14–16). Some are so oblique that they meet in the middle line dorsally and ventrally (Text-fig. 16). Where the bothridia narrow posteriorly the radial muscles become less extensive (Text-figs. 17, 18) and finally disappear as such when the free portions of the bothridia merge into the scolex. A few delicate radial muscles now appear in the lateral region of the scolex (Text-fig. 18) and are found, also, in the cephalic peduncle sparsely distributed around the peripheral zone.

(4) *Longitudinal muscles.* The longitudinal muscles are unevenly distributed in the scolex. There are a few isolated fibres, scattered through the parenchyma, which pass on into the proliferating zone. The longitudinal muscles in the scolex are best developed near both lateral margins dorsally and ventrally at the point where the bothridia join the scolex. Here they form four separate bundles of longitudinal fibres, two dorsal and two ventral, which extend throughout the length of the scolex (Text-figs. 12–19). They are inserted into the basement membrane near the tip of the scolex and are not very conspicuous in the anterior half. In the posterior half and opposite each of these bundles is a series of obliquely placed muscle fibres extending from the dorsal and ventral wall of each bothridial face, on either side, backwards and inwards to join the relevant bundle which consequently consists of an increasing number of fibres. The four bundles continue through the cephalic peduncle lying close to the body wall and on into the proliferating zone where the constituent fibres become more delicate and are eventually absorbed into the superficial longitudinal muscles of the body wall (Text-fig. 19).

The concerted action of all these muscles in the scolex can bring about a variety



Text-figs. 17–21. *Ditrachybothridium macrocephalum* gen.nov., sp.nov.

Text-fig. 17. Transverse section through the scolex, showing decrease in prominence of the bothridia, change in direction of the bothridial nerves as they arise, the branching of the excretory vessels and the poorly developed musculature.

Text-fig. 18. Transverse section through the posterior end of the scolex, showing further narrowing of the bothridia and consequently more prominent lateral region of scolex.

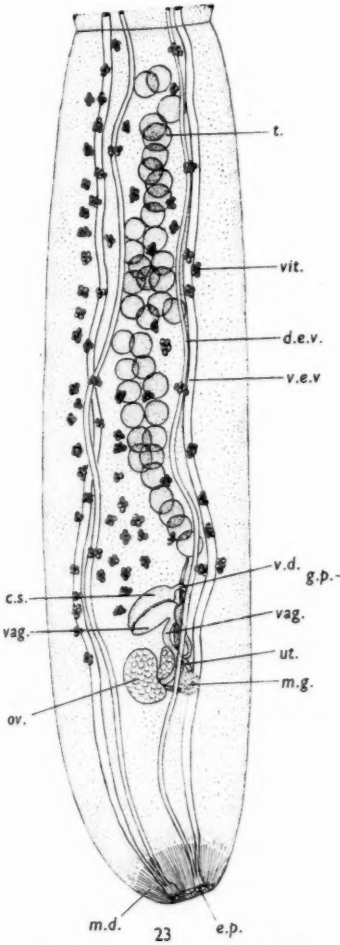
Text-fig. 19. Portion of sagittal section through margin of bothridium, showing bundle of longitudinal muscles.

Text-fig. 20. Transverse section through neck, showing very weakly developed muscles, excretory vessels, lateral nerves and the cells filling a large part of the parenchyma.

Text-fig. 21. Excretory pores in terminal segment, ventral view.

of movement in different directions, such as a lengthening and shortening of the scolex and a curling over and increase in concavity of the bothridia.

Strobila. The musculature of the strobila is very weakly developed. Superficial circular and longitudinal muscles are present just below the body wall. In the proliferating zone there are some scattered transverse, dorso-ventral and radial fibres, but as the reproductive organs develop the muscles seem to disappear.



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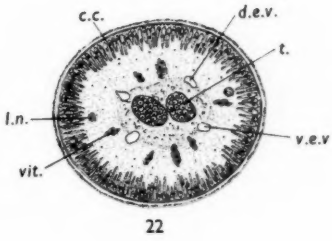

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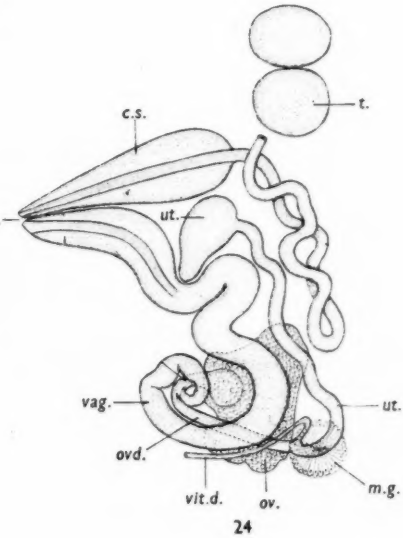

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Text-figs. 22–24. *Ditrachybothridium macrocephalum* gen.nov., sp.nov.

Text-fig. 22. Transverse section through almost mature segment in the testicular region, showing testes in centre with ring of seven yolk follicles surrounding them, excretory vessels, lateral nerves and cuticle-secreting cells.

Text-fig. 23. Last segment of complete worm, dorsal view, showing reproductive system, excretory vessels and terminal excretory pores.

Text-fig. 24. Reconstruction of genitalia, from left side, not fully mature. Left lobe of ovary omitted.

There is no division into cortex and medulla, but there is a deep layer of cells below the body wall throughout the scolex and strobila, presumably the cuticle-secreting cells (Text-figs. 12, 20, 22 and 25–27). The central region of most of the strobila is occupied by parenchyma which is denser immediately around the reproductive organs (Text-figs. 22, 25–27). In the proliferating zone and early part of the strobila there are numbers of cells in the central zone.

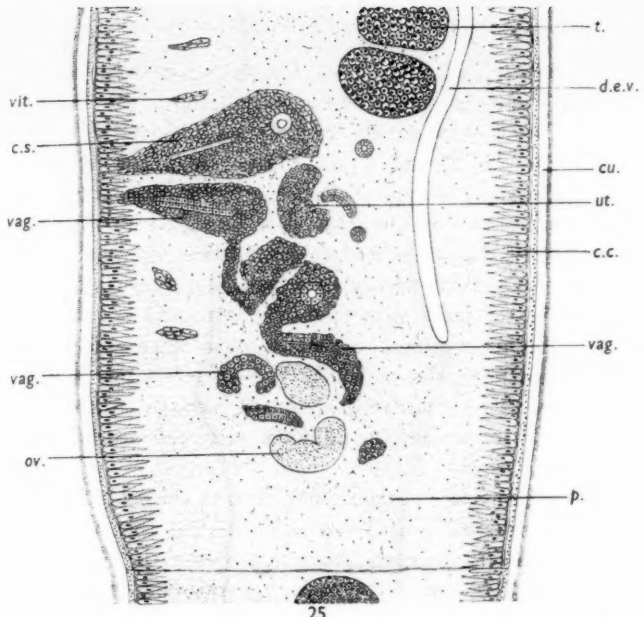
Nervous system

The nervous system is simple due to the simplicity in structure of the scolex and bothridia. It consists of a nerve centre or brain at the apex of the scolex, a lateral nerve on either side passing backwards through the scolex and into the strobila, bothridial nerves to the bothridia, anterior nerves to the anterior extremity and posterior nerves to the central zone of the scolex behind the brain (Text-figs. 1, 2).

The brain consists of two rounded cerebral ganglia placed very near together immediately behind the apical organ (Text-figs. 1–3, 13). They are connected together near their anterior borders by a short transverse commissure. No ring commissure could be found. From the anterior end of each ganglion arises a dorsal and a ventral nerve. These four nerves pass forwards and outwards for a short distance when each divides into two (Text-fig. 1), an anterior nerve passing forwards around the apical organ and a posterior nerve passing backwards. The latter could only be traced for a short distance, but probably passes into the central zone of the scolex behind the brain. There are therefore four anterior and four posterior nerves supplying the anterior region of the scolex itself (Text-figs. 1, 2 and 11). The lateral nerves arise, one on either side, from the posterior margin of each ganglion and continue throughout the length of the scolex, diverging gradually from one another as they enter the strobila, and extend to the posterior extremity (Text-figs. 1, 2 and 14–18). Arising from each lateral nerve in the scolex are sixteen pairs of bothridial nerves supplying the bothridia. These are evenly distributed along the length of the bothridia from a short distance behind the brain (Text-figs. 1, 2). The dorsal and ventral nerves of each pair on either side pass obliquely outwards into the free lateral regions of the bothridia (Text-figs. 1, 15). As the scolex widens and the bothridia narrow the direction taken by the bothridial nerves alters slightly. They turn more dorsally and ventrally respectively until they are at right angles to the lateral nerve and finally turn still further until they are directed obliquely towards the middle line, thus following the curve of the lateral margins of the bothridia on to the dorsal and ventral surfaces of the scolex (Text-figs. 1, 2 and 17). Two of the bothridial nerves, numbers 13 and 14, were found to divide each into two branches, one to the central zone of the bothridium and the other to the diminishing free zone (Text-figs. 1, 2 and 17). This is the region where the longitudinal muscle band is best developed. Branching was not observed in any of the other bothridial nerves but may possibly occur.

Excretory system

There is a dorsal and a ventral excretory vessel on either side, extending throughout the scolex and strobila (Text-figs. 2, 3 and 23). The ventral vessel is



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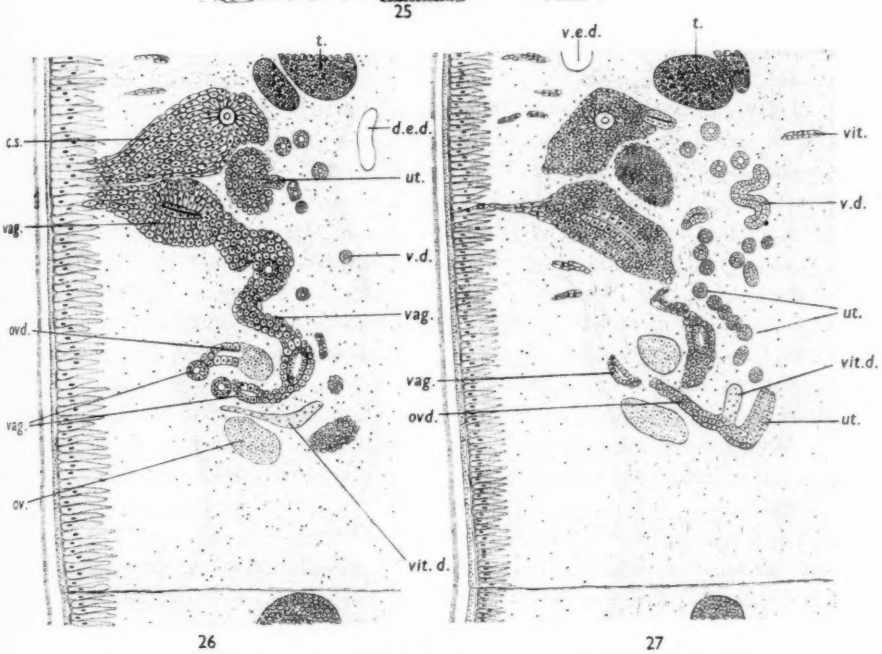

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Text-figs. 25–27. *Ditrachybothridium macrocephalum* gen.nov., sp.nov.

Text-fig. 25. Sagittal section through the centre of an almost mature segment, showing cirrus sac, vagina, ovary, portion of uterus, yolk follicles and testes.

Text-fig. 26. Sagittal section through the same segment, to the right of the previous, showing the same structures, the oviduct arising from the median portion of the ovary and the median yolk duct.

Text-fig. 27. Sagittal section through the same segment, to the right of the previous, showing the same structures and also the vas deferens, oviduct, yolk duct opening into oviduct, and origin and continuation of the uterus.

slightly larger in diameter than the dorsal. In the strobila the vessels are conspicuous and pursue a sinuous course throughout, lying to the inner side of the lateral nerve and in mature segments dorsally and ventrally to most of the genitalia (Text-figs. 20, 22, 23 and 25–27). They are not connected with one another. The excretory pore is terminal (Text-fig. 23) or, due to unequal contraction of the muscles of the body wall, may come to lie subterminally on the ventral surface (Text-fig. 21). As the dorsal and ventral vessels approach the end of the last segment, they unite to form a very short common excretory vessel on either side. These two short vessels then open separately into a shallow depression at the tip of the segment. This depression is a permanent structure around the margin of which the longitudinal muscles of the body wall converge to form almost a sucker-like structure, so that the size of the depression can be controlled. The lining of this shallow cavity is raised into papilliform processes around the excretory pores themselves (Text-fig. 21).

The dorsal and ventral vessels extend forwards into the scolex at the posterior end of which they are situated, as in the strobila, to the inner side of the lateral nerves (Text-figs. 3, 17 and 18). Passing forwards, however, they come to lie dorsally and ventrally to them (Text-figs. 3, 15 and 16). Anteriorly they pass forwards above and below the cerebral ganglia and on either side of the apical organ the dorsal unites with the ventral vessel on each side, forming a loop (Text-figs. 2, 3 and 11). There is no connexion between the vessels of the right and left sides until a short distance behind the cerebral ganglia. Throughout most of the scolex there is a complicated network of small excretory vessels uniting the two dorsal and the two ventral vessels respectively (Text-figs. 2, 3 and 17; Pl. V, fig. 3). This network is usually one layer deep, but in places may be double and there may be an anastomosis between the dorsal and ventral networks in the central zone (Text-fig. 17). Both vessels, on either side, are also connected with one another at frequent intervals by dorso-ventral branches to the inner side of the lateral nerves (Text-figs. 2, 17). Branches are also given off from the dorsal and ventral vessels to the bothridia in which they subdivide (Text-figs. 2, 3). The whole scolex, therefore, is permeated by a network of excretory vessels.

There is the same fundamental simplicity and repetition in the excretory system as in the nervous system and musculature due to the simplicity and to the uniformity of structure throughout the length of the scolex and bothridia.

Reproductive system

With the exception of the last segment in the longest worm the genitalia are not fully mature. Material for investigating the system has, therefore, been somewhat inadequate and sectioned specimens though showing the arrangement of the male and female glands and their ducts does not show them in their completed form.

The genital atrium opens in the mid-ventral line in the posterior quarter of the segment (Text-figs. 23, 25–27). The actual opening was not yet formed in the sectioned specimens (Text-figs. 25–27).

Male genitalia. There are from fifty-two to sixty-two testes in each segment. The number was less in the last segment of the complete worm shown in Pl. V,

fig. 5, and Text-fig. 23, but this seems to be an exception. They are arranged in a row along the centre extending from just in front of the genital atrium to the anterior margin (Text-fig. 23). The row may be one, two or occasionally three testes deep and they are confined to the area between the four excretory vessels (Text-figs. 22, 23 and 25). The testes are rounded or slightly oval and measure 0·060 × 0·049 to 0·068 × 0·044 mm. They are first recognizable as such at about one-quarter of the way down the strobila. The vas deferens is a long coiled tube 0·014 mm. in diameter. It extends from the level of the last testis backwards in an undulating manner in the middle line, dorsally to the female reproductive organs, as far as the anterior end of the ovary (Text-figs. 23, 24, 26 and 27). Here it turns forwards in a narrow U-shaped right-hand bend to form the ascending limb which passes forwards to just behind the last testis (Text-fig. 24). It then turns ventrally to enter the cirrus sac. The cirrus sac occupies nearly half the depth of the segment and is almost at right angles to the ventral body wall, the proximal end being slightly anterior to the distal end (Text-figs. 23, 24 and 25–27). It measures 0·17 mm. in length in a segment 0·341 mm. in diameter, and is pear-shaped with a maximum breadth, proximally, of 0·06 mm. As the specimens are immature these measurements cannot be taken as final. The vas deferens enters the cirrus sac and continues as a straight tube throughout its length. There is as yet no apparent differentiation into cirrus, ductus ejaculatorius or receptaculum seminis and the cirrus sac consists mainly of an undifferentiated mass of cells.

Female genitalia. The ovary is very slightly towards the ventral side of the segment and lies not far behind the genital atrium or a little more than half-way between the last testis and the posterior margin of the segment (Text-figs. 23, 24 and 25–27). It is bilobed, each lobe measuring 0·136 mm. in length and 0·073 mm. in breadth. The two lobes are joined by a short median portion (Text-figs. 23, 24). The oviduct arises from the ventral side of the median portion (Text-figs. 24, 26) and is joined shortly after its origin by the vagina. The vagina opens to the exterior in the mid-ventral line close behind the cirrus sac. The actual opening was not formed, but its position was clearly evident (Text-figs. 25, 26). From the external opening the vagina continues inwards for a short distance more or less at right angles to the ventral body wall, it then turns backwards, taking an undulating course towards the ovary (Text-figs. 24, 25–27). It passes between the right and left lobes of the ovary dorsally to the median portion and turns below the latter towards the ventral side of the segment (Text-figs. 24, 26). Opposite the origin of the oviduct it describes almost a complete circle and opens into the oviduct (Text-figs. 24, 25 and 26). The vagina throughout most of its length measures 0·117 mm. in diameter, but is wider near the external opening. At this stage in development the wall consists of a layer of large cells with prominent nuclei. Near the opening the wall is several layers thick. The oviduct, having received the vagina, passes postero-dorsally behind the median portion of the ovary to the left of and at first slightly in front of the vagina (Text-figs. 24, 27). Just beyond the dorsal margin of the ovary it receives on its anterior side a median yolk duct which comes from the ventral region of the segment passing just posterior to the vagina and between it and the oviduct (Text-figs. 24, 26 and 27). It could

only be traced for a short distance, but is presumably formed by the union of smaller yolk ducts from the yolk follicles. Just beyond the point where the yolk duct enters the oviduct is Mehlis's gland (Text-figs. 23, 24 and 26), a spherical mass of cells 0·007 mm. in diameter surrounding the oviduct in this region. The uterus emerges from Mehlis's gland, turns abruptly forwards and now lies dorsally to the rest of the female genitalia. It continues forwards (Text-figs. 24, 27), turning slightly ventrally to pass below the vas deferens and dorsal to the vagina. It is a tube measuring 0·012 mm. in diameter which is about the same as the vas deferens but less than the vagina. The uterus ends blindly in a fairly large mass of cells situated between the proximal ends of the cirrus sac and vagina (Text-figs. 24, 25–27). There is no uterine pore. The uterus may develop into a sac or an elongated tube for storing eggs as the segment becomes mature and eventually gravid.

The yolk glands are follicular and are scattered in the parenchyma around the remainder of the genitalia, there being up to seven or eight in a single transverse section (Text-figs. 22, 23). They extend from the anterior margin of the segment to the anterior border of the ovary, though they are not as common behind the level of the testes as they are in the testicular region. They measure 0·031 × 0·017 mm. to 0·050 × 0·025 mm., the longer axis radiating outwards from the centre of the segment. Each consists of a group of small cells. No yolk ducts other than the median yolk duct could be found.

No eggs were available for examination and there is no indication of the life-cycle.

Ditrachybothridium *gen.nov., diagnosis*

Scolex consists of head proper and short cephalic peduncle; two oval flattened or spoon-shaped sessile bothridia, one dorsal and one ventral; bothridia armed along border and on outer convex face with rows of small, backwardly directed spines; cephalic peduncle unarmed, terminated by a velum; apex of scolex bluntly pointed and with feebly developed apical organ; neck present; strobila acraspedote, cylindrical with sides and lines of division between segments straight; posterior segments four or five times longer than broad; excretory pores paired, terminal, surrounded by a muscular depression; brain consists of two cerebral ganglia joined by a short transverse commissure, a series of evenly distributed pairs of bothridial nerves arise from the lateral nerves in the scolex; cirrus sac and vagina open in mid-ventral line in posterior quarter of segment, cirrus immediately in front of vagina; cirrus sac large, pyriform, occupying half the depth of the segment, vas deferens long and coiled; testes 52–62, in row, 1 to 2 deep, in centre of anterior two-thirds of segment; ovary bilobed, posterior; yolk glands inconspicuous, diffuse, surrounding most of remainder of genitalia as far back as anterior end of ovary; no uterine pore; in spiral valve of elasmobranch fishes.

Type species Ditrachybothridium macrocephalum *gen.nov., sp.nov., with the characters of the genus, from the spiral valve of* Raja fullonica L., R. circularis *Couch and* Scyliorhinus caniculus (L).

As regards its systematic position, *Ditrachybothridium macrocephalum* gen.nov., sp.nov., seems to be most closely related to the genus *Echinobothrium* van Beneden.

It resembles this genus in that the scolex consists of a head proper bearing two bothridia, one dorsal and one ventral, followed by a cephalic peduncle. In *E. typus* van Beneden (1849), *E. musteli* Pintner (1889) and *E. brachysoma* Pintner (1889) the bothridia are armed with small spines. The shape of the segments is similar to that described by Southwell (1925) for *E. boissii* (= *E. typus* van Beneden), namely, '...first much broader than long, becoming square, then cylindrical. The sides and division lines of the segments are perfectly straight.' The musculature of the strobila is very weakly developed in both genera. The genital pore is in the mid-ventral line in the posterior part of the segment. The cirrus sac is large and the vas deferens a long tube either bent on itself or convoluted. The arrangement of the testes is the same, there being two parallel rows in the centre of the anterior two-thirds of the segment. The vagina opens immediately behind the cirrus and in *E. mathiasi* Euzet (1951) the terminal part, as in *Ditrachybothridium macrocephalum*, is pyriform. The ovary is posterior and bilobed and there is no uterine pore. The uterus in *Echinobothrium* may be sacciform or tubular and presumably will take one of these forms in the gravid segments of the present species. The yolk glands are diffuse and inconspicuous and consist in both of rosette-shaped groups of cells. The worms occur in the spiral valve of elasmobranch fishes. *E. typus* van Beneden and *E. affine* Diesing have been recorded from *Raja fullonica*, one of the hosts in which *Ditrachybothridium macrocephalum* is found.

The differences between *Ditrachybothridium* and *Echinobothrium* are less numerous. The cephalic peduncle is unarmed in the former, but armed in the latter with very characteristic spines and there are two groups of prominent spines at the apex of the scolex. These are the two most distinctive features of the genus *Echinobothrium*. Most of the species of *Echinobothrium* are small, measuring up to 6 mm. in length, although *E. longicolle* Southwell (1925) measures up to 20–30 mm. There are fewer testes in *Echinobothrium*, the largest number of about 25 to 30 being found in *E. mathiasi* Euzet (1951) and *E. longicolle*. These latter are, however, minor differences. The greatest differences occur in the details of the scolex and the greatest similarities in the arrangement of the genitalia.

The seven species of *Echinobothrium* constitute the order Diphyllidea Carus, an order which is recognized by many helminthologists but not by all. Southwell (1925) created an order, the Heterophyllidea, to include *Echinobothrium* and four other genera which he characterized as follows: 'Head very variable in appearance, it does not bear either four suckers, four bothridia, four proboscides or two bothria.' Wardle & McLeod (1952) grouped *Echinobothrium* with eight other *genera inquirenda* which cannot be fitted with ease into the orders Tetraphyllidea, Lecanicephala or Disculiceptidea, though they seem to have some affinities for these orders. So the fate of *Echinobothrium* and consequently of the order Diphyllidea is not fully determined, but whatever the outcome *Ditrachybothridium* should stand somewhere near the genus *Echinobothrium*.

If the order Diphyllidea is to remain and if it is to include *Ditrachybothridium* the definition of the order will need to be amended to something like the following: cestodes in which the scolex is formed of a head proper and a cephalic peduncle. The scolex is provided with two foliaceous or spoon-shaped bothridia, one dorsal

and one ventral, usually armed with small spines and sometimes divided each into two by a median longitudinal ridge. The apex of the scolex may bear an insignificant unarmed apical organ or a large rostellum bearing dorsal and ventral groups of hooks. The cephalic peduncle may be short and unarmed or long and armed with several longitudinal rows of large T-shaped hooks with their points directed backwards. The strobila is cylindrical, acraspedote and weakly muscular, the posterior segments are longer than broad. The genital pore is mid-ventral in the posterior part of the segment. The testes are in a row 1 to 2 deep in the centre of the anterior half or so of the segment. The bilobed ovary is posterior and the yolk glands lateral or surrounding the rest of the genitalia in the region in front of the ovary. No uterine pore, uterus tubular or sacciform. Adults in the intestine of elasmobranch fishes.

In the distribution of the yolk glands *Ditrachybothridium macrocephalum* more nearly resembles the Tetrarhynchidea and the Pseudophyllidea, where the yolk glands are in the cortical parenchyma surrounding all the other organs. The Tetrarhynchidea are parasites of Selachians, and in *Nybelinia* and *Tentacularia* it is interesting to note that the genital atrium is displaced on to the ventral surface. The genital atrium is often on the ventral surface in the Pseudophyllidea and the scolex, with its dorsal and ventral bothria, bears a superficial resemblance to that of *Ditrachybothridium macrocephalum*.

D. macrocephalum is evidently one of those forms which does not fit precisely into any existing order.

SUMMARY

1. *Ditrachybothridium macrocephalum* gen.nov., sp.nov., is described.

2. The systematic position is discussed and the species compared with species of the genus *Echinobothrium* to which it seems to be fairly closely related.

I should like to express my thanks to Mr S. Prudhoe, of the British Museum (Natural History), for having examined the present specimens and for his comments on them.

REFERENCES

BAYLIS, H. A. (1950). A review of the species of *Dinobothrium* (Cestoda), with a description of a new species. *Parasitology*, **40**, 96–104.

BENEDEN, P. J. VAN (1849). Notice sur un nouveau genre d'helminthe cestode. *Bull. Acad. Belg. Cl. Sci.* **16**, 182–93.

EUZET, L. (1951). *Echinobothrium Mathiasi* n.sp. (Cestode Diphyllidea) parasite d'une raie. (*Leiobatis aquila* L.). *Bull. Soc. Zool. Fr.* **76**, 182–8.

PINTNER, TH. (1889). Neue Untersuchungen über den Bau des Bandwurmkörpers. 1. Zur Kenntnis der Gattung *Echinobothrium*. *Arb. zool. Inst. Univ. Wien*, **8**, 371–420.

SOUTHWELL, T. (1925). A monograph on the Tetraphyllidea with notes on related cestodes. *Liverpool School of Tropical Medicine Memoir* (N.S.), no. 2, 368 pp.

WARDLE, R. A. & MCLEOD, J. A. (1952). *The Zoology of Tapeworms*, Minnesota Press, 780 pp.

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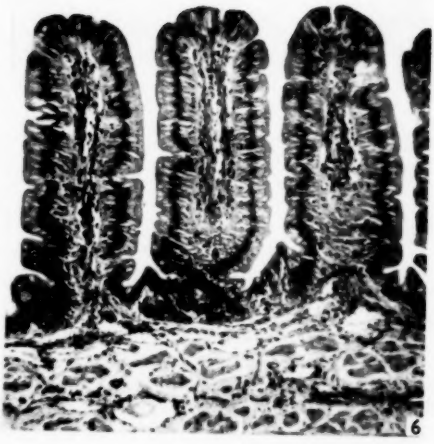

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Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
genitali
Fig. 5.
Fig. 6.

a.l.
a.n.
a.o.
b.
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c.c.
c.g.
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cu.
d.e.v.
d.v.m.
e.n.s.
e.p.
g.p.
l.m.
l.n.
m.d.

EXPLANATION OF PLATE V

(Photomicrographs by D. Hemingway Jones, B.Sc., to whom I am most grateful.)

Ditrachybothridium macrocephalum gen.nov., sp.nov.

Fig. 1. Entire worm.
Fig. 2. Scolex, dorsal or ventral view, showing spines along margin of bothridium.
Fig. 3. Contracted scolex, showing some of the excretory vessels.
Fig. 4. Last segment of complete worm (fig. 1), showing testes and immature female genitalia.
Fig. 5. Last segment of another complete worm, showing mature genitalia (see Text-fig. 23).
Fig. 6. Vertical section through wall of spiral valve of *Raja fullonica* to show villi.

KEY TO LETTERING OF FIGURES

(All drawings are semi-diagrammatic)

a.l.	anterior loop of excretory vessel	*m.g.*	Mehlis's gland
a.n.	anterior nerve	*n.*	neck
a.o.	apical organ	*ov.*	ovary
b.	bothridium	*ovd.*	oviduct
b.n.	bothridial nerve	*p.*	parenchyma
c.c.	cuticle-secreting cells	*p.n.*	posterior nerve
c.g.	cerebral ganglion	*r.m.*	radial muscles
c.s.	cirrus sac	*s.*	scolex
cu.	cuticle	*sp.*	spine
d.e.v.	dorsal excretory vessel	*t.*	testis
d.v.m.	dorso-ventral muscles	*t.m.*	transverse muscles
e.n.s.	excretory network in scolex	*ut.*	uterus
e.p.	excretory pore	*v.*	velum
g.p.	genital pore	*v.e.v.*	ventral excretory vessel
l.m.	longitudinal muscles	*vag.*	vagina
l.n.	lateral nerve	*v.d.*	vas deferens
m.d.	muscular depression around excretory pores	*vit.*	yolk follicle
		vit.d.	yolk duct

(*MS. received for publication* 30. IV. 1958—Ed.)

MORPHOLOGICAL AND TAXONOMIC STUDIES ON MAMMALIAN TRYPANOSOMES

IX. REVISION OF *TRYPANOSOMA DIMORPHON*

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(With 5 Figures in the Text)

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I. INTRODUCTION

Although members of the *Congolense* group are the most important pathogenic trypanosomes affecting domestic animals in tropical Africa, there is still some uncertainty regarding the number of species belonging to this group. If we disregard those names which are undoubtedly synonyms of other species (e.g. *Trypanosoma nanum*, *T. pecorum*, *T. suis*, etc.), there remain two species which are generally recognized as the only valid representatives of this group, viz. *T. congolense* and *T. simiae*. However, while the diagnostic features of the latter are now well defined (Hoare, 1936*a*, *b*, 1957; Culwick & Fairbairn, 1947), there are considerable morphological and biological differences among various strains attributed to *T. congolense*.

Such strains vary in their host-parasite relations (including range of hosts) and virulence, as well as in clinical manifestations, while in a number of cases it has been shown that the morphology of the trypanosomes does not conform to that of typical *T. congolense*. It is therefore conceivable that the diversity in the biological characters of some strains is correlated with morphological differences. If this were proved, exact criteria would be available for the differential parasitological diagnosis of infections caused by distinct strains of *congolense*-like trypanosomes and for their taxonomic treatment, which are matters of practical importance for the elucidation of animal trypanosomiases in Africa.

In this connexion it should be noted that from time to time there have appeared

reports of the presence, in some strains, of trypanosomes which were considerably longer than those of typical *T. congolense* (cf. Hoof, 1924; Hoof, Henrard & Peel, 1948; Schwetz, 1931, 1938; Hoare, 1958), and resembled *T. dimorphon* Laveran & Mesnil, 1904. As the latter species has never been adequately described, its name was frequently misused, and has gradually passed out of circulation, so that at present no one recognizes the independence of *T. dimorphon*, which is regarded merely as a synonym of *T. congolense*. However, in view of the reported existence of strains apparently conforming to *T. dimorphon*—which have also been encountered by me—it would seem that the merging of this species in *T. congolense* might have been premature.

This question could, of course, be settled by a comparative study of diverse strains of *congolense*-like trypanosomes, but unfortunately (as will be shown below) in the case of *T. dimorphon* no exact criteria are available for its identification. The only alternative would be to make a revision of this species on the basis of the original material studied by Laveran and Mesnil. Although it seemed highly improbable that this material was still available and in a suitable condition for a morphological investigation, I approached Professor E. Roubaud, of Institut Pasteur, Paris, on this matter. He responded immediately, by kindly placing at my disposal the collection of original films made by Laveran and Mesnil during the first decade of this century. On examination of these preparations I found, to my great surprise, that many of the films were in an excellent state of preservation, showing perfectly stained trypanosomes. In fact, their appearance in preparations made half a century ago compares favourably with that of trypanosomes in well-stained recent slides. Thus, through the courtesy of Professor Roubaud and thanks to the faultless technique of Laveran and Mesnil, I was able to undertake a revision of *T. dimorphon* based on the type material.

II. HISTORY

Before proceeding with the revised description of *T. dimorphon*, it is necessary to clear up some misconceptions about this species that have prevailed for many years, until eventually this name was suppressed. It should be noted, however, that there were no convincing objective grounds for its abolition: it was rather an act of desperation in face of the existing confusion regarding the identity and nomenclature of this species, which hitherto remained unsolved.

Since the present paper is devoted to the strain of *T. dimorphon* described by Laveran and Mesnil, it is essential that its relation to other trypanosomes attributed to this species should be clearly defined. With this end in view, I have consulted the literature—especially the earlier publications—on these trypanosomes, and found these works so full of discrepancies, misinterpretations and misstatements, that it seemed almost impossible to disentangle the resulting confusion. However, a critical perusal of the original papers—interpreted in the light of our present knowledge of pathogenic African trypanosomes—has enabled me to reduce to some order the chaotic state into which *T. dimorphon* has fallen, as will be seen from the brief outline of its history, which follows.

The trypanosome in question was first recorded by Dutton & Todd (1903) from horses in Gambia. Under the name 'The Gambian Horse Trypanosome', they gave a composite description of the parasites found in ten horses, which were said to comprise the following three types: (*a*) a very small 'tadpole-shaped' form, measuring 11–13 μ in length and possessing a short free flagellum; (*b*) a long form, measuring 26–30 μ in length and possessing a long free flagellum; and (*c*) a short stumpy form, 16–19 μ long, with a very short free flagellum. All these forms of what appeared to be a polymorphic trypanosome were regarded by the authors as belonging to one (unnamed) species. However, from the description and the accompanying figures, there can be no doubt that Dutton and Todd were dealing with a mixed infection, consisting of a *congolense*-like trypanosome (their 'tadpole-shaped' forms) and *T. brucei*. And indeed, the authors themselves likened the 'long' and 'stumpy' forms to *T. gambiense*. This was also the view held by Bruce, Hamerton, Bateman, Mackie & Bruce (1910*b*), Laveran & Mesnil (1912) and Bruce (1914), while Yorke (1914) suggested that, in Dutton and Todd's cases, *T. congolense* was accompanied by *T. vivax*, because previously Yorke & Blacklock (1911) found a mixed infection of these trypanosomes in a Gambian horse.

A strain of Dutton and Todd's equine trypanosome was sent to the Liverpool School of Tropical Medicine, from which an infected rat was supplied to Laveran and Mesnil, at the Institut Pasteur of Paris. This strain was studied by them and described as *T. dimorphon* (Laveran & Mesnil, 1904*a*), representing a dimorphic trypanosome of the *congolense* type, devoid of a free flagellum. The publication of Laveran and Mesnil's paper was the starting-point of all the controversy in the literature concerning this trypanosome, since the descriptions given by Dutton and Todd, on the one hand, and by Laveran and Mesnil, on the other, differed in important points. This discrepancy was further aggravated by the fact that the name *T. dimorphon* was applied by many writers indiscriminately to the trypanosomes described both by the British and French authors. Actually there is no evidence in the paper by Dutton & Todd (1903) that they had ever called it otherwise than 'The Gambian Horse Trypanosome', though, according to Laveran & Mesnil (1904*a*, 1912), it would seem that Dutton and Todd did suggest the name *T. dimorphon* to their collaborator, Dr H. E. Annett. He mentioned it in a letter to one of the French authors, who adopted it for the trypanosome described by them, and even credited it to Dutton and Todd (Laveran & Mesnil, 1904*a*, *b*). Whatever the case may be, since the name *T. dimorphon* was first published by Laveran and Mesnil, according to the *Rules of Zoological Nomenclature* it should be attributed to them. The amendment to that effect was actually made in the second edition of their monograph (Laveran & Mesnil, 1912).

The cause of the discrepancy in the descriptions of the Gambian trypanosome is explained in a memoir by Thomas & Breinl (1905), from which it is evident that the strain, studied by them in Liverpool and by Laveran and Mesnil in Paris, originated from a single horse (viz. Dutton and Todd's Case VI) sent to them from Gambia. They state that: 'In no single instance has the long form, described by Dutton and Todd and possessing...a long flagellum, been seen; in this we are in accord with Laveran and Mesnil.' Further information was provided by

Montgomery & Kinghorn (1909*a*), according to whom Dutton and Todd were dealing with two distinct trypanosomes in Gambian horses: one of them was polymorphic (as described by Dutton & Todd, 1903, chiefly from their Case I), and 'was never employed in Europe', while the other, which had the characters described by Laveran and Mesnil for their *T. dimorphon*, originated from Dutton and Todd's Case VI. These facts were later confirmed by Laveran & Mesnil (1912). However, Montgomery and Kinghorn recognized the independence of both Dutton and Todd's 'Gambian Horse Trypanosome' (from Case I), for which they reserved the name *T. dimorphon* (*sensu* Dutton & Todd, 1903), and of Laveran and Mesnil's trypanosome (from Dutton and Todd's Case VI), for which they proposed the new name *T. confusum*. But, as rightly pointed out by Mesnil (1909) and by Laveran & Mesnil (1912), there was no justification for these changes, first because by priority the name *T. dimorphon* should be retained for the species described by Laveran and Mesnil, secondly because the name *T. confusum* was preoccupied by an avian trypanosome (Lühe, 1906).

From the foregoing account it is seen that the identity of *T. dimorphon* had been fairly well established, in contrast to that of Dutton and Todd's 'Gambian Horse Trypanosome', which represented a mixture of *T. brucei* and a *congolense*-like trypanosome. Nevertheless, Dutton and Todd's description of a distinct polymorphic trypanosome continued to be accepted by many authors, who reported the occurrence of so-called '*T. dimorphon*' in strains with the combined characters of a *congolense*-like form and long trypanosomes with a pronounced free flagellum, which obviously belonged to other species. Thus, Dutton, Todd & Kinghorn (1907) recorded the finding, in various ungulates from the Congo, of what was evidently a mixed infection of the small trypanosome and *T. vivax*. From the description and figures of strains from Rhodesian ruminants, given by Montgomery & Kinghorn (1908, 1909*b*), it is evident that they were also dealing with mixed infections, the concomitant species in cattle being *T. brucei* and that in sheep, *T. vivax*. Similarly, as already noted by Donizio (1921), the trypanosomes described by Martoglio (1911) from livestock in Italian Somaliland, under the names *T. somalilense* (= *somaliense*) and *T. cellii*, also represented mixed infections with *T. brucei*. Lastly, in some cases the name *T. dimorphon* was apparently given to pure infections with *T. brucei* (Balfour, 1906, 1909; Kopke, 1908; Mendes, Mora, Monteiro & Bruto da Costa, 1909). It is also interesting to note that Kinghorn, Yorke & Lloyd (1913) described from a bushbuck a new species, *T. multiforme*, which was unmistakably a mixed infection with trypanosomes corresponding to the three forms seen by Dutton and Todd.

The question now arises whether the 'tadpole' forms present among Dutton and Todd's 'polymorphic' equine trypanosomes represented *T. dimorphon* (*sensu* Laveran & Mesnil, 1904*a*) or *T. congolense*. Unfortunately it is impossible to determine their identity, for trypanosomes conforming to these forms in structure and size are common to both species. The same uncertainty applies to mixed infections attributed to *T. dimorphon* by other authors.

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III. ORIGINAL DESCRIPTION

The historical review given above served to establish the identity of *T. dimorphon* as a strain isolated in 1903 from a single Gambian horse (Dutton and Todd's Case VI) and studied by Laveran & Mesnil (1904*a*). It was also demonstrated that the status of the other equine strains observed in Gambia, representing mixed infections of *T. brucei* with undetermined trypanosomes of the *Congolense* group, remains uncertain. As far as *T. dimorphon* is concerned, therefore, those strains can be ignored, leaving only the species described and named by the French workers for further consideration. Since the present study is based on Laveran and Mesnil's original material, and in order to appreciate the points in which my revised description differs from or amplifies Laveran and Mesnil's account, it will be necessary first to summarize the data concerning *T. dimorphon* recorded in their publications.

According to Laveran & Mesnil (1904*a*, *b*, 1912), *T. dimorphon* is represented by trypanosomes varying in size and comprising two distinct forms: (*a*) short ones, measuring 10–15 μ in length, and (*b*) long ones, the average length of which is 22 μ but may reach 25 μ. In the short forms the posterior end of the body is rounded, while in the long ones it is typically pointed or conically truncated, but may also be rounded. In both forms the undulating membrane is never well developed, though it is somewhat more pronounced in the long ones. None of these trypanosomes has a free flagellum, and its absence was regarded as a constant characteristic of this species. Although the two forms are said to be connected by intermediate ones, the short one was not regarded as a young stage of the long one, because stages of division were observed in both. The appearance of the trypanosomes—conforming to the above description—is depicted in a series of figures drawn by Laveran. In their monograph, Laveran & Mesnil (1912) placed *T. dimorphon* together with *T. congolense*, *T. pecorum* and *T. nanum*, as a natural assembly corresponding to the present *Congolense* group. As is known, however, the differential characters, which at that time separated the last three species (especially lack of cross-immunity between the respective infections), were later invalidated, with the result that *T. pecorum* and *T. nanum* were merged in *T. congolense* as synonyms. According to Laveran and Mesnil, the other species (now *T. congolense*) differ from *T. dimorphon* in the complete absence of long forms measuring upwards of 22 μ in length. But, since in some infections with *T. dimorphon* long forms are rare and because *T. congolense* closely resembles the short forms of the latter species, *T. dimorphon* may sometimes be mistaken for *T. congolense*.

The effect of infections attributed to *T. dimorphon* upon its hosts was described by a number of authors, but—for reasons already stated—the only reliable data on the host-parasite relationship of this trypanosome are those that concern the strain isolated from Dutton and Todd's Case VI. From the accounts given by Dutton & Todd (1903) and Thomas & Breinl (1905) it is seen that the horse in question was under observation—in Gambia and England—for about 2½ years, during which a chronic infection persisted without any serious clinical mani-

festations attributable to trypanosomiasis. Similarly, in a horse inoculated with *T. dimorphon* by Laveran & Mesnil (1912) the course of infection was chronic, persisting for 7 months, without the animal's health being affected, and terminating in spontaneous recovery. In two goats infected by the same observers the results were variable: in one the infection was acute, terminating fatally within a fortnight, whereas in the other the course was chronic, lasting about 2 years before the animal died. Sheep infected by Laveran recovered, but a sheep infected by Thomas & Breinl (1905) died after 21 weeks. According to the last-named authors and Laveran & Mesnil (1912), in experimentally infected dogs the disease runs an acute and rapidly fatal course. Lastly, laboratory rodents used by the French and British workers proved to be highly susceptible to infection, the course of which may be acute or chronic.

From a comparison of the virulence of *T. dimorphon* and *T. congolense*, Laveran & Mesnil (1912) concluded that the former species produced a more acute infection in rodents and a more severe disease in sheep and goats than the latter species. Moreover, Thomas & Breinl (1905) have shown that *T. dimorphon* was infective to baboons (*Cynocephalus* [= *Papio*] *sphinx*), which are known to be refractory to *T. congolense* and other trypanosomes.

Laveran & Mesnil (1912) believed that *T. dimorphon* has a very wide geographical distribution throughout tropical Africa: in fact, they regarded it as one of the most common pathogenic trypanosomes in that continent. However, since the records quoted by them include many strains of doubtful identity, this conclusion cannot be accepted unreservedly.

IV. MATERIAL

As mentioned before, the material for the present revision consisted of the original preparations made by Laveran and Mesnil, which were kindly placed at my disposal by Professor E. Roubaud. This collection contains over forty blood films, stained by one of the Romanovsky methods. Among them there were only about a dozen slides in which the trypanosomes—though recognizable—were too faded for a morphological study, but in about 50 % the staining was in a perfect state of preservation. In addition to these preparations, I examined a film with this trypanosome from the collection of the late Dr C. M. Wenyon.

The blood films in question were taken from various animals in which the strain was maintained in Paris from 1903 to 1909. Particulars about the different passages are given on the labels attached to the slides, while further information about some of them was traced by me in Laveran and Mesnil's publications. The present study was therefore based on the type material of *T. dimorphon*, comprising samples used by these authors up to 1904 (when they first described this species) and those from subsequent passages of the same strain. Since these authors did not designate the sample containing the type specimens, the material used for the revised description is based on the examination of syntypes, which are equivalent to holotypes (cf. Frizzell, 1933).

The present study was restricted to the earlier years (1903–7) of the life of this

strain, and a selection was made of the best preparations, representing samples taken from different passages, particulars of which are given below.

Material used in present work

No.	Sample	Slide labelled
1	*A*	'Rat 1: 15 Nov. 1903'
2	*B*	'Rat 1: 19 Nov. 1903'
3	*C*	'Rat 1: 20 Nov. 1903'
4	*D*	'Rat 3: 25 Nov. 1903'
5	*E*	'Mouse 3: 5 Dec. 1903'
6	*F*	'Mouse 3: 8 Jan. 1904'
7	*G*	'Goat A: 6 Jan. 1904'
8	*H*	'Goat B: 6 Jan. 1904'
9	*J*	'Mouse 2: 19 Apr. 1904'
10	*K*	'Mouse: 22 Apr. 1906'
11	*L*	'Mouse 104: Nov. 1907'

Remarks. According to Laveran & Mesnil (1912), the strain of *T. dimorphon* studied by them was received from Liverpool at the end of 1903, in a rat infected with trypanosomes isolated from a horse (Dutton and Todd's Case VI). Presumably, therefore, 'Rat 1' (= samples *A*, *B*, *C*) was actually the animal in question, representing the 1st passage from the original host. The infections in goats (= samples *G*, *H*) are fully described by Laveran & Mesnil (1904*a*, 1912, pp. 601–2). Sample *K* is a film made by Dr Wenyon from the same strain in 1906, while working at the Institut Pasteur, Paris.

V. REVISED DESCRIPTION

(*a*) *The problem*

One of the reasons why *T. dimorphon* was suppressed and absorbed by *T. congolense* was the incomplete description given by Laveran & Mesnil (1904*a*, *b*; 1912), who did not provide all the necessary data for its exact identification and differentiation from the other species of the *Congolense* group. The diagnosis of these closely related species presents many difficulties, because, on the one hand, the trypanosomes in question have a number of morphological features in common, and, on the other, the characters distinguishing them may be absent in some of the random samples examined. However, in the case of *T. simiae* which is sufficiently well defined, this difficulty can be overcome by examination of samples taken at different phases of the infection, some of which usually reveal all the typical forms.

The main distinguishing characteristic of *T. dimorphon*, according to Laveran and Mesnil, is its dimorphism, expressed in the presence of (*a*) short forms which are indistinguishable from typical *T. congolense*, and (*b*) long ones exceeding the length of any trypanosomes encountered in the latter species (for measurements see p. 214). However, these authors gave no indication of the number of trypanosomes measured by them, of their length distribution and means, or of variation between samples. Nor did they produce any objective evidence of the alleged dimorphism of the species in question. Without these particulars, Laveran and Mesnil's data are too meagre to be of any diagnostic or statistical value.

The object of the present revision was, accordingly, to verify and amplify the

original description of *T. dimorphon*, in order to provide all the mensural and morphological data for determining its status and its relation to other members of the *Congolense* group.

(*b*) *Biometry*

In order to determine the exact measurements of *T. dimorphon*, I have carried out a biometrical investigation of the eleven samples of the strain listed above. As in my previous papers, the length of the trypanosomes was measured by drawing (with the aid of a camera lucida at $\times 2500$) a line running through the middle of the body of each trypanosome, from one end of the body to the other, and measuring this line with a divider set at 0·05 in. ($= ca.\ 0{\cdot}5\mu \times 2500$). An attempt was also made to ascertain, by the method devised by Fairbairn, Culwick and Willett (described by Hoare, 1956), whether the length distributions of *T. dimorphon* could be resolved into distinct curves corresponding to the two forms described by Laveran and Mesnil, thereby settling the question of its alleged dimorphism. However, examination of the numerous trypanosomes measured by me did not reveal any clear-cut morphological differences by which the two forms could be separated into distinct groups. It was therefore impossible to classify each trypanosome individually, as in the case of the polymorphic trypanosomes of the *Brucei* group (cf. Hoare, 1956). Nevertheless, in all the trypanosomes measured by me certain structural details were noted, in case they might be correlated with differences in body-length.

Table 1. *Frequency distribution of lengths and statistical constants of* 11 *samples of* Trypanosoma dimorphon

	Length (μ)														Mean length			Number
mples	11	12	13	14	15	16	17	18	19	20	21	22	23	24	(μ)	σ	$2\sigma_M$	measured
A	—	—	5	12	15	22	11	16	13	4	2	—	—	—	16·4	1·9	0·4	100
B	—	—	8	5	9	11	23	22	14	6	2	—	—	—	16·9	1·9	0·4	100
C	—	1	7	4	6	8	15	19	17	15	5	3	—	—	17·5	2·3	0·5	100
D	—	—	2	8	8	9	18	20	13	10	4	7	0	1	17·6	2·3	0·5	100
E	—	1	5	8	13	18	21	16	10	6	1	1	—	—	16·5	2·0	0·4	100
F	—	—	6	10	19	17	24	14	7	3	—	—	—	—	16·1	1·7	0·3	100
G	1	5	13	12	17	21	17	8	4	1	0	1	—	—	15·3	2·0	0·4	100
H	1	1	10	8	18	19	23	12	7	1	—	—	—	—	15·8	1·8	0·3	100
J	—	—	8	9	9	18	16	25	12	3	—	—	—	—	16·5	1·9	0·4	100
K	4	6	14	29	32	31	31	21	18	5	5	1	2	1	15·9	2·4	0·3	200
L	1	8	12	29	23	16	7	3	1	—	—	—	—	—	14·4	1·6	0·3	100
Total	7	22	90	134	169	190	208	176	116	54	19	13	2	2	16·2	—	—	1200

The statistical data for the measurements of the lengths of trypanosomes in the eleven samples of *T. dimorphon* are given in Table 1, while in Fig. 1 they are represented graphically by the Dice-Leraas-Davis method (cf. Hoare, 1956). In this chart the range of lengths is shown by horizontal lines and the means are indicated by cross-bars. On either side of the mean, the limits of the black rectangles correspond to the standard deviation of the series (σ), those of the white rectangles to twice the standard deviation of the mean ($2\sigma_M$). The statistical significance of the difference between the means of the samples is assessed by the degree of overlap or separation of the corresponding rectangles. Thus, when the white rectangles of two samples just do not overlap (i.e. meet end to end), the probability that they are samples of the same population is (*a*) only 0·02, provided that the standard deviations are the same and the

samples are of equal size (more than thirty individuals), and (*b*) not more than 0·05, if the standard deviation of one is smaller than that of the other, or the samples are of different sizes. Therefore if—in any set of samples compared— the white rectangles do not overlap, there is reasonable probability that the differences in the measurements are statistically significant, their significance increasing with the distance between these rectangles. The significance of the difference between two samples is still greater when the black rectangles neither overlap nor are separated (indicating that 84 % of the specimens in such samples would be separable): in such cases the difference between the corresponding populations can be regarded as being of subspecific order.

In Fig. 1 the samples are arranged in chronological order of the animal passages through which the strain was maintained in the course of four years (1903–7).

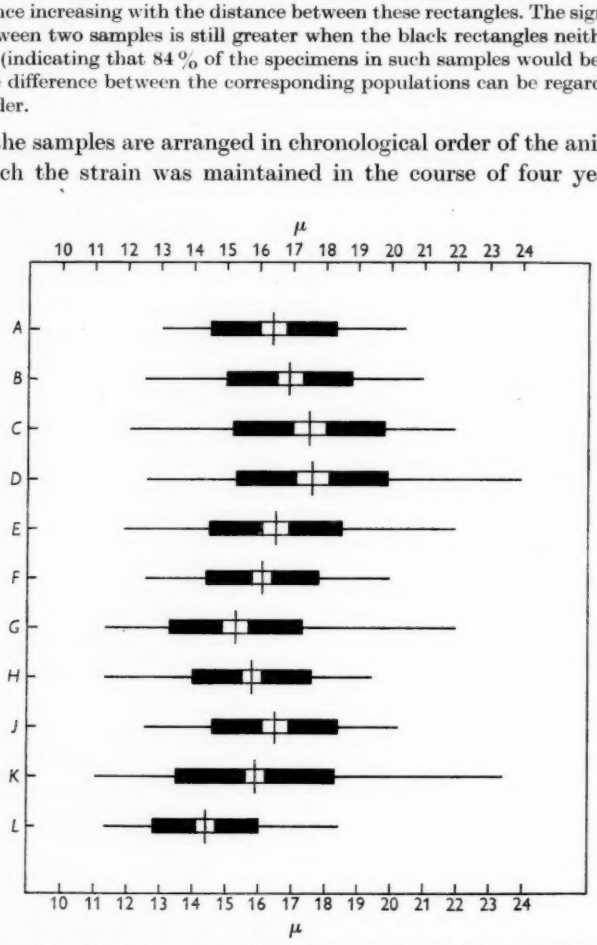

Fig. 1. *T. dimorphon*. Variation in length of eleven samples (*A–L*, cf. Table 1). Range of lengths is shown by horizontal lines, means by cross-bars; on either side of the mean, black rectangles represent standard deviation of the series (σ) and white rectangles represent twice the standard deviation of the mean ($2\sigma_M$).

It can be seen at a glance that during that time the mean lengths of the trypanosomes, in samples examined at different periods, varied considerably, the variation being particularly demonstrable when the samples are compared in the ascending order of their means, viz. $L = 14{\cdot}4$, $G = 15{\cdot}3$, $H = 15{\cdot}8$, $K = 15{\cdot}9$, $F = 16{\cdot}1$, $A = 16{\cdot}4$, J and $E = 16{\cdot}5$, $B = 16{\cdot}9$, $C = 17{\cdot}5$, $D = 17{\cdot}6$. In this series the means show a gradation from $14{\cdot}4\,\mu$ at one end to $17{\cdot}6\,\mu$ at the other, the difference between these extremes—when assessed on the basis of the degree of separation

between the white rectangles—being statistically highly significant. In considering the possible factors responsible for this wide variation, one might suspect the influence of the host upon the size of the trypanosomes. However, it is evident from the chart that samples from the same host-species differ as much as those from diverse hosts. Thus, the means for trypanosomes from individual mice (*L*, *K*, *F*, *E*, *J*) vary from 14·4 to 16·5 μ, while those from goats ($G = 15{\cdot}3\,\mu$, $H = 15{\cdot}8\,\mu$) fall within the same range. As regards the trypanosomes from rats (*A*–*D*), though the upper limit of their means (17·6 μ) is higher than in the other animals, the lower limit (16·4 μ) is also within the range of the trypanosomes from mice. The most striking example of variation is encountered in samples *A* and *C*, in which the difference between the means (16·4 and 17·5 μ respectively) is statistically highly significant, in spite of the fact that they were taken at an interval of only 5 days from one and the same host (Rat 1).

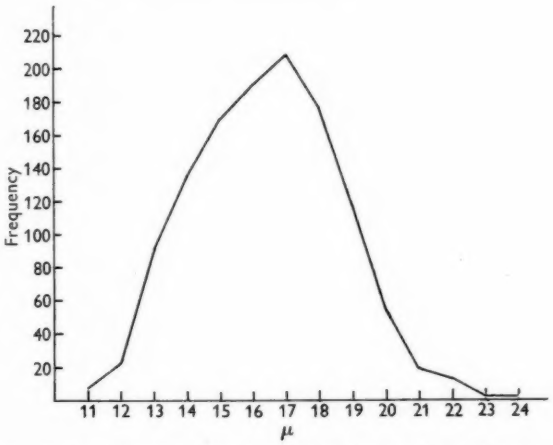

Fig. 2. *T. dimorphon*. Length distribution for the total (= 1200) measurements in eleven samples.

From these results it is evident that the length of *T. dimorphon* varies considerably from sample to sample, the difference in the mean length between some samples being statistically significant. Hence it follows that the measurement of a random sample does not necessarily provide constant quantitative criteria for its specific identification. It may even be misleading, as in the case of sample *L*, in which the dimensions of the trypanosomes correspond so closely to those of *T. congolense* (see Table 4, p. 226) that it could easily be mistaken for that species. However, a true mensural characteristic of *T. dimorphon* can be arrived at by pooling and plotting the lengths of all the samples, thereby smoothing out the differences between them. The result of this operation is given in Fig. 2, representing a curve for the grand total of 1200 counts, which shows a normal length distribution, with a range from 11 to 24 μ, and mode = 17 μ (mean = 16·2 μ: see Table 1).

We now turn to the question of dimorphism in *T. dimorphon*, which—as stated

above—could not be determined on purely morphological grounds. However, it was thought that dimorphism—if present—might be revealed in curves showing the frequency distribution of lengths in the eleven samples measured. When these were plotted, it was found that in the case of some samples (cf. Table 1 and Fig. 3) the curves appeared to be bimodal. In order to ascertain whether this diversity was due to dimorphism of the trypanosomes, my friend Dr J. C. Broom has kindly carried out a statistical analysis of my data, and writes as follows:

From the means and standard deviations obtained from the measurements (Table 1), calculations were made for each sample of the frequency distributions that would be expected if the samples were drawn from populations—with these parameters—in which the lengths are normally distributed. The observed and expected distributions were compared by means of the χ^2 test, and the probability (P) that the differences between the distributions could arise as the result of chance are shown in Table 2.

Table 2. *Probability that samples of* Trypanosoma dimorphon *are derived from normally distributed populations*

Sample	P	Sample	P
A	0·2	*G*	$>0{\cdot}05$
B	0·1	*H*	0·1
C	0·02	*J*	0·01
D	0·3	*K*	0·9
E	0·8	*L*	0·4
F	0·3		

When $P = 0{\cdot}01$, differences as great or greater could be expected in 1 of 100 tests, and when $P \leqslant 0{\cdot}01$, the differences are regarded as being statistically significant, i.e. that they are unlikely to be the result of chance. On this criterion it may be assumed that sample *J* ($P = 0{\cdot}01$) was not drawn from a normally distributed population, while sample *C* ($P = 0{\cdot}02$) may be regarded as a borderline case. A similar problem, encountered in the case of *T. evansi*, was subjected to statistical analysis by Trevan ('Appendix' in Hoare, 1956), who reached the conclusion that some at least of the samples of morphologically indistinguishable forms comprised individuals derived from more than one normally distributed population. He considered that probably physiological factors were responsible for the differences and suggested that the heterogeneity might be due to the presence of crops of trypanosomes of different ages. It seems not unreasonable to postulate that the divergences from normal distributions observed in the present series are due to similar factors, and this hypothesis is particularly plausible in the case of sample *C*, which was taken from the same rat which provided samples *A* and *B* on earlier days (see Fig. 3).

From Dr Broom's analysis it is seen that the great majority of samples (nine out of eleven) represent homogeneous populations with normal length distributions, only two providing evidence of a certain degree of heterogeneity. The weight of evidence is therefore against any dimorphism of *T. dimorphon*, which can be regarded as a monomorphic species.

(*c*) *Morphology*

The morphology of *T. dimorphon* was studied in the 1200 specimens measured for statistical purposes, in each of which the following features were specially recorded: (*a*) existence of a free flagellum, (*b*) shape of posterior end of the body, (*c*) position of the nucleus, (*d*) position of the kinetoplast.

In general, the structure of *T. dimorphon* conforms to the pattern seen in other members of the *Congolense* group, especially *T. congolense*, from which it differs mainly in its greater length (Fig. 4). One of the characteristic features of the flagellum is its relation to the kinetoplast. While in the longer forms the intracytoplasmic portion of the axoneme usually starts in front of the kinetoplast

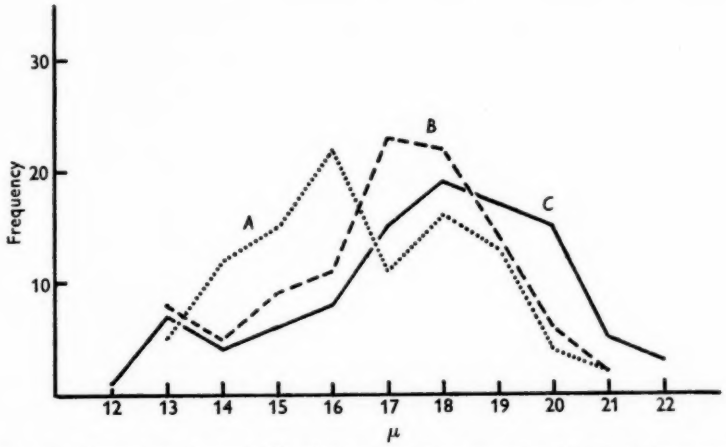

Fig. 3. *T. dimorphon.* Length distributions in three samples (*A*, *B*, *C*) from rat 1.

(Fig. 4, *h*, *j*, *l–s*), in many of the shorter ones it arises somewhat behind the kinetoplast (Fig. 4, *a–f*, *i*, *k*). The latter arrangement seems to be peculiar to this group of trypanosomes. The flagellum terminates at the anterior end of the body without projecting beyond it: there is thus no free flagellum in *T. dimorphon*. The body is slender, its undulating membrane is weakly developed and is not thrown into conspicuous folds. The posterior end of the body is either rounded (Fig. 4, *a–c*, *e–j*) or drawn out (Fig. 4, *d*, *l–o*, *q–s*), in the latter case being usually obtusely pointed or somewhat truncated. In order to ascertain whether its shape is correlated with the size of the trypanosomes, the percentages of forms with rounded and pointed ends were plotted against the lengths of the trypanosomes. The result is shown in two curves (Fig. 5, *PEr*, *PEp*), which indicate a definite tendency for the posterior end to be drawn out as the trypanosomes increase in length. The position of the nucleus was determined by the distance of its centre from the anterior and posterior ends of the body. If the distance from both ends was equal, the nucleus was regarded as being in the middle; if it was nearer to one of the ends, it was taken to be in the corresponding part of the body. According to this criterion, the nucleus of *T. dimorphon* usually lies in the middle or in the posterior part of the body. There is no correlation between its position and the length of the body in trypanosomes shorter than 13 μ, but in the longer forms (Fig. 5, *Np*, *Nm*) the nucleus tends to shift backwards as the length of the trypanosomes increases.

However (as seen from the curves in Fig. 5) there is a gradual transition from trypanosomes with rounded to those with pointed ends, on the one hand, and from those with nuclei in the middle and posterior parts of the body, on the other.

Therefore, as already stated, these characters cannot serve to separate the trypanosomes into two groups, corresponding to the short and long forms described by Laveran & Mesnil (1904*a*, 1912), and they provide no evidence of dimorphism in *T. dimorphon*.

As in other *congolense*-like trypanosomes, the kinetoplast of *T. dimorphon* is of medium size, viz. larger than in the *Brucei* group and smaller than in the *Vivax* group (cf. Hoare, 1957). Its position in the body varies in different samples (Fig.4): in a small minority (1–5 %) it is terminal, but in the great majority (95–99 %) it is

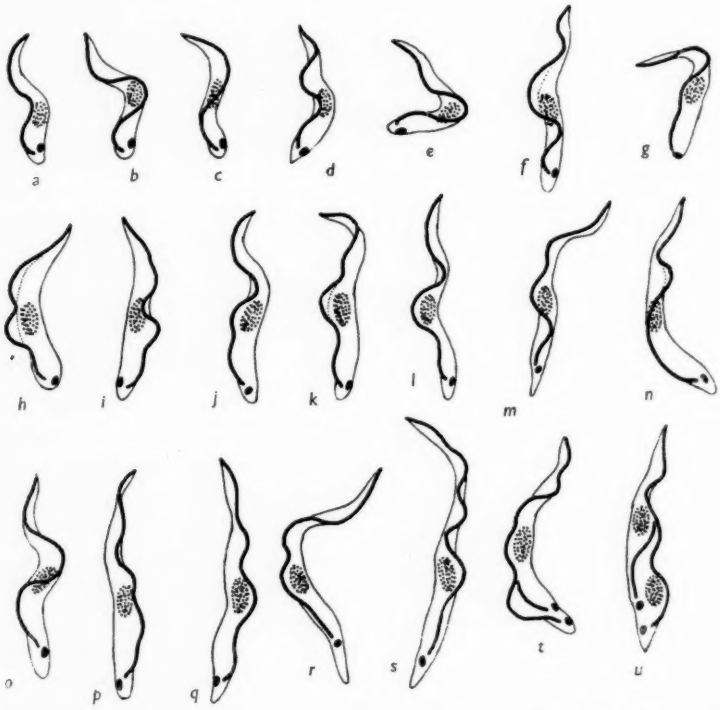

Fig. 4. *T. dimorphon*. *a–s*: trypanosomes arranged in ascending order of lengths; *t*, *u*: dividing forms. (Camera lucida drawings, at × 2000.)

subterminal, being situated at a distance up to 3 μ from the posterior end. Moreover, most of the subterminal kinetoplasts (54–100 %) occupy a marginal position, at the edge of the body. There appears to be no correlation between the position of the kinetoplast and the length of the body, though in long trypanosomes with a pointed posterior end it is more frequently situated further from this end than in short forms. Multiplication apparently takes place chiefly among the larger flagellates (Fig. 4, *t*, *u*), for among 100 dividing trypanosomes observed in the course of this study 90 % were long forms and only 10 % were short.

Diagnosis. From the preceding biometrical and morphological study, the diagnostic characters of *T. dimorphon* can be summarized as follows. In general,

this species is represented by trypanosomes measuring from 11 to 24 μ in length, with a mean of 16·2 μ, but there is considerable variation in the mean lengths of different populations (14·4–17·6 μ), even in samples taken at various intervals from the same individual host. The body of the trypanosomes is slender, its undulating membrane is not pronounced, and there is no free flagellum. The posterior end of the body is rounded (chiefly in short forms) or pointed (chiefly in long forms), while the nucleus lies in the middle or in the posterior part of the body. The kinetoplast is fairly large, and is typically subterminal and marginal. Although certain characters seem to be correlated with the length of the trypanosomes, there is no evidence of a clear-cut dimorphism, therefore *T. dimorphon* can be regarded as being monomorphic.

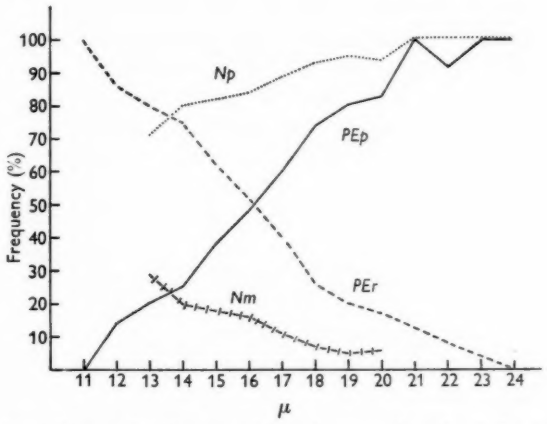

Fig. 5. *T. dimorphon*. Curves showing correlation between length of the trypanosomes and (1) shape of the posterior end of the body (*PEr*: rounded; *PEp*: pointed); (2) position of the nucleus (*Nm*: in middle of the body; *Np*: in posterior part of the body).

(*d*) *Previous records*

The equine strain from Gambia, first described by Laveran and Mesnil and revised in the present paper, is actually the only authentic record of *T. dimorphon*, but on *a priori* grounds there can be no doubt that this species has a much wider geographical and hostal distribution. And indeed, as already mentioned in the Introduction, a number of authors had drawn attention to the presence in some *congolense*-like strains of unusually long trypanosomes, which they attributed to *T. dimorphon*. Although most of the published data do not supply precise clues to the identity of these strains, we can now assess their value more accurately in the light of the present revision.

One of the earliest records was by Martin (1906), who attributed all the infections detected by him in domestic animals of French Guinea to *T. dimorphon*, and in fact the measurements (13–23 μ) and illustrations of the trypanosomes found by him in sheep, goats, pigs and dogs—but not in bovines and equines—conform to this species. Later Martin, Leboeuf & Roubaud (1909) reported the occurrence of *T. dimorphon* in livestock of French Congo (now French Equatorial Africa), but

both the dimensions (10–15 μ, 12–17 μ) and figures given by them indicate that the trypanosome in question was probably *T. congolense*. Likewise, a trypanosome isolated by Edington (1908) from a horse in Zanzibar and studied by Bruce, Hamerton & Bateman (1909) was thought to be *T. dimorphon*, but the measurements (10·4–17·1 μ, mean 13·6 μ) suggest that they too were dealing with *T. congolense*. It is curious therefore that Laveran & Mesnil (1912) also believed that the Zanzibar strain might have been *T. dimorphon*. Among the trypanosomes found by Jowett (1910) in cattle of Portuguese East Africa and referred to *T. dimorphon*, only those isolated from his 'Ox 48' agree with this species in size (11–24 μ) and appearance (his Fig. 3), whereas the trypanosomes in other animals were undoubtedly *T. congolense*. It is also highly probable that the bovine trypanosome from Southern Rhodesia described by Bevan (1910*a*, *b*) was *T. dimorphon*, to which its size (15–30 μ, with 18–20 μ predominating) and appearance correspond. Fry (1911) recorded trypanosomes from domestic animals of Anglo-Egyptian Sudan under the name '*T. pecorum* or *nanum*' (now *T. congolense*) but, judging from his fig. 7 and from its size (mean 15·7 μ), the trypanosome from sheep might have been *T. dimorphon*. It would also seem that this species was correctly identified by Yorke & Blacklock (1911) in one of the horses (*B*) observed in Gambia, while the short forms isolated from a double infection in another horse (*A*), though attributed by Blacklock (1912*a*, *b*) to *T. dimorphon*, evidently represented *T. congolense* (for its length see Table 4). Martoglio (1911) gave the name *T. somalilense* to a strain from livestock in Italian Somaliland, in which there was obviously a double infection with *brucei*-like forms and a trypanosome with the characters of *T. dimorphon* (length 11–26 μ; his figs. 6, 8, 18). Delanoë (1914) was probably right in classifying a bovine trypanosome from the Ivory Coast (measuring 13–20 μ, mean 16 μ) as *T. dimorphon*. Among the trypanosomes of domestic animals, which Curson (1928) recorded from Zululand under the name *T. congolense*, are depicted (his pl. II, fig. 1*b*) short and long forms from an ox that closely resemble *T. dimorphon*. Other observers (Hoof, 1924; Schwetz & Fornara, 1929; Schwetz, 1931, 1934, 1938; Hoof & Henrard, 1933; Hoof *et al.*, 1948) recognized the existence in the Belgian Congo—especially in cattle—of a longer variety of *T. congolense*, which might be *T. dimorphon*. Finally, a trypanosome recently isolated from a dog in Nigeria by Godfrey (1958) was tentatively referred to *T. dimorphon*, but its mean lengths (range 14·5–15·7 μ) are shorter than those found by me in this species.

It would thus seem that strains suggestive of *T. dimorphon* had probably been observed in the following localities and hosts: Gambia (horses), French Guinea (sheep, goats, pigs, dogs), Ivory Coast (cattle), Belgian Congo (cattle), Sudan (sheep), Somalia (livestock), Southern Rhodesia (cattle), Portuguese East Africa (cattle), Zululand (cattle), and possibly Nigeria (dog).

VI. SYSTEMATIC POSITION

(*a*) *Differential diagnosis*

From the foregoing account it is evident that *T. dimorphon* has all the general attributes of the *Congolense* group. However, since certain features are common to all the species of this group and some populations of a given species do not contain all the characteristic forms, it may be difficult to identify the trypanosomes in random samples. In order to determine the systematic position of *T. dimorphon* and to differentiate it from the other species, it is necessary to consider the characters of *T. simiae* and *T. congolense*.

As is known (Hoare, 1936*a*, *b*; Culwick & Fairbairn, 1947), *T. simiae* is a polymorphic species, comprising short *congolense*-like forms, and long forms, among which the majority (so-called standard *simiae* forms) are stout and have a well-developed undulating membrane, while others (so-called *rodhaini* forms) are slender and have an inconspicuous undulating membrane; moreover, some of the long forms may have a free flagellum. The differential characters of this species and *T. dimorphon* are given in Table 3.

Table 3. *Comparison of* Trypanosoma simiae *and* T. dimorphon

Species	*T. simiae**	*T. dimorphon*†
Morphism:	Polymorphic	Monomorphic
Length (μ) Range	12·5–24	11–24
Length (μ) Means	17·0–18·2	16·2
Percentage of forms		
(1) Standard *simiae*	88·8–92·2	None
(2) Slender (*rodhaini*)	5·2–7·8	58–81
(3) Typical *congolense*	2·0–4·2	19–42
(4) With free flagellum	0·8–3·9	None

* Data taken from Hoare (1936*a*, *b*).

† Data based on examination of 100 trypanosomes in each of following samples: *A*, *D*, *E*, *H*, *J* (total = 500).

From this table it is seen that, while the lengths of *T. dimorphon* and *T. simiae* are similar, they differ markedly in other respects. Thus the standard forms (showing a superficial resemblance to thin forms of *T. brucei*), which are predominant in *T. simiae*, are completely absent in *T. dimorphon*, whereas the typical *congolense* forms and the slender *rodhaini* forms, which constitute a small minority in *T. simiae*, are the commonest forms found in *T. dimorphon*. Furthermore, a free flagellum has never been seen in the latter.

In general, the structure of *T. dimorphon* does not differ from that of *T. congolense*, as I was able to satisfy myself in the case of a porcine strain of the last-named species from the Belgian Congo. In preparations which were kindly provided by the late Dr L. van Hoof, I measured 400 trypanosomes (100 in each of four samples), noting in each specimen the same characters that were recorded by me for *T. dimorphon*. As in the latter, the posterior end of the body in *T. congolense* was either rounded or drawn out, and there was a similar tendency for this

end to be more pointed as the trypanosomes increased in length. As regards the nucleus, in addition to positions in the middle and posterior parts of the body, in a fair number of *T. congolense* (12–36 %) the nucleus was situated in the anterior part. As in *T. dimorphon*, the kinetoplast of *T. congolense* was in most cases subterminal and marginal, but there was a higher proportion of trypanosomes (4–30 %) in which it was terminal. Some of the characters of *T. congolense* observed by me in a single strain were also described by a number of previous authors. As the structural differences between *T. dimorphon* and *T. congolense* are quite negligible, the comparison of these two species can be restricted to mensural characters.

Table 4. *Comparison of* Trypanosoma congolense *and* T. dimorphon

	Length (μ)		
Authors	Range	Means	Species
C. A. H.	8–17	12·2	*T. congolense*
Bruce *et al.* 1910*a*	8–18	13·3	
Blacklock, 1912*b*	9–19·5		
Kinghorn *et al.* 1913	9·5–19	13·6	
Bruce *et al.* 1913	9–18	13·9	
Bruce *et al.* 1913	9–18	14·0	
Bruce *et al.* 1913	11–18	14·3	
Kinghorn *et al.* 1913	10–19	14·4	
C. A. H.	11·3–22	15·3	*T. dimorphon*
C. A. H.	11·3–19·5	15·8	
C. A. H.	11–23·5	15·9	
C. A. H.	12·5–20	16·1	
C. A. H.	13–20·5	16·4	
C. A. H.	12·5–20·3	16·5	
C. A. H.	11·8–22		
C. A. H.	12·5–21	16·9	
C. A. H.	12–22	17·5	
C. A. H.	12·5–24	17·6	

According to the original description of *T. congolense* by Broden (1904), this trypanosome measured from 10·5 to 16 μ in length, the means for two strains being 12·5 and 13 μ. Although there is now a vast literature on this species, I propose to consider only the relatively few records in which the measurements had been treated statistically, to which I have added the results of a biometrical study of the porcine strain mentioned above. Among the measurements of *T. dimorphon* those of sample *L* have been omitted for the following reason. It is a well-known fact that in various species of trypanosomes the proportion of different forms present in some populations is subject to fluctuation. In the case of *T. dimorphon*, it has been pointed out by Laveran & Mesnil (1912)—and confirmed by Curasson (1943)—that the long forms may occasionally disappear, leaving only short forms in the population, which can then be mistaken for pure *T. congolense*. There can be little doubt that sample *L* of *T. dimorphon*, whose range (11·3–18·5 μ) and mean (14·4 μ) correspond to the measurements of *T. congolense*, is an example of this phenomenon.

As this atypical sample is not representative of the species to which it belongs, it can be safely ignored. The measurements of the two species in question are compared in Table 4.

It is evident from this table that these two species are clearly separable by their lengths. Thus, the total ranges of *T. congolense* (8–19·5 μ) and *T. dimorphon* (11–24 μ), though overlapping, are quite distinct, while the difference between their means (12·2–14·4 and 15·3–17·6 μ respectively) is highly significant. The means of each of these species form a continuous group, without a real break in the variation of length between the respective strains and/or samples, but there is a definite gap between the highest mean of the shorter species (14·4 μ) and the lowest mean of the longer one (15·3 μ). Here it may be added that, since the qualitative morphological characters of *T. congolense* and *T. dimorphon* are similar, the shorter forms depicted in Fig. 4 (*a–k*) can also serve to illustrate the appearance of *T. congolense*.

Apart from the difference in mensural characters, there is some evidence that *T. dimorphon* is more virulent than *T. congolense* (see p. 215).

(*b*) *Classification*

In the preceding section it has been shown that *T. dimorphon* can be distinguished from *T. simiae* by a number of morphological features (Table 3), while it differs from *T. congolense* mainly in the range of means, the two species falling into two distinct groups, whose mean measurements do not overlap. The relation between *T. congolense* and *T. dimorphon* is thus comparable to that of *T. uniforme* and *T. vivax* (cf. Hoare & Broom, 1938). As the result of the present revision, the differential diagnosis of *T. dimorphon* is so well defined that it should now be removed from the synonymy of *T. congolense* and restored to its original status as an independent species. However, the occurrence of strains with intermediate mensural characters—such as recorded by Godfrey (1958)—cannot be excluded. If their existence is confirmed, it might be necessary to reduce the rank of *T. dimorphon* and other *congolense*-like strains to some minor taxon (e.g. subspecies or race). In a previous publication (Hoare, 1957) I have already tentatively included *T. dimorphon* in the *Congolense* group, as an independent species characterized by dimorphism, but in the light of the present work the diagnosis of this group should be amended as follows.

III. Congolense *group*

Monomorphic or polymorphic forms. Free flagellum absent or present. Kinetoplast medium, typically marginal. Development in *Glossina*: midgut and proboscis.

(*a*) Monomorphic (free flagellum absent or short, undulating membrane inconspicuous). (1) Short forms (means 12·2–14·4 μ): *T. congolense*. (2) Long forms (means 15·3–17·6 μ): *T. dimorphon*.

(*b*) Polymorphic (short forms without free flagellum; and long forms, comprising stout ones with conspicuous undulating membrane and slender ones with inconspicuous undulating membrane, free flagellum absent or present): *T. simiae*.

Finally, we turn to the nomenclature of *T. dimorphon*, in which are reflected the changing views on the status of this species since it was first described nearly 55 years ago.

Trypanosoma dimorphon Laveran & Mesnil, 1904

Synonyms

(1) 'The Gambian Horse Trypanosome', Dutton & Todd, 1903 (*pro parte*).
(2) [*Non*] *Trypanosoma dimorphon* Dutton & Todd, 1903, *auctorum*.
(3) *Trypanozoon dimorphon* (Laveran & Mesnil, 1904) Lühe, 1906 (*pro parte*).
(4) *Trypanosoma confusum* Montgomery & Kinghorn, 1909 (*non T. confusum* Lühe, 1906).
(5) *T. pecorum* Bruce *et al.*, 1910 (*pro parte*).
(6) *T. somalilense* Martoglio, 1911 (*? pro parte*).
(7) *T. congolense* var., Schwetz, 1938.
(8) *T. congolense* [*auctorum* (*non* Broden, 1904), *pro parte*].

VII. SUMMARY

Owing to inadequate and conflicting descriptions, there have always been doubts about the identity of *Trypanosoma dimorphon*, which is commonly regarded as a synonym of *T. congolense*. However, the occurrence of atypical strains, with characters like those of *T. dimorphon*, has re-opened the question of the possible independence of this species.

To solve this problem, the author has undertaken a complete revision of *T. dimorphon*, based on Laveran and Mesnil's original type-material. In the course of this work, a total of 1200 trypanosomes, belonging to samples taken from animal passages in the course of 4 years, were studied biometrically and morphologically.

It was shown that the structure of *T. dimorphon* is comparable to that of *T. congolense*, but it differs from the latter in greater length, its range being 11–24 μ, while the means vary in different populations from 15·3 to 17·6 μ. Although certain characters (e.g. shape of posterior end of the body and position of nucleus) seem to be correlated with the length of the trypanosomes, there is a gradual transition between the different forms. Moreover, when examined statistically, the great majority of samples proved to be homogeneous populations with normal length distributions. Therefore, in the absence of any evidence of dimorphism, *T. dimorphon* is regarded as a monomorphic species.

In a review of previous records, strains which had been erroneously attributed to *T. dimorphon* have been separated from those conforming to this species, which probably has a fairly wide distribution in Africa.

From a comparison of *T. dimorphon* with other species of the *Congolense* group, it is seen that it can be distinguished from *T. simiae* by a number of morphological features, while it differs from *T. congolense* mainly in the range of mean lengths, the two species falling into two distinct groups, whose mean measurements do not overlap.

It is concluded that the present revision provides grounds for the removal of

T. dimorphon from the synonymy of *T. congolense* and its restoration to the original position as an independent species. The characteristics of the *Congolense* group have been amended in accordance with this change in its composition.

I am much indebted to Professor E. Roubaud for the loan of the historical material without which the revision of *T. dimorphon* could not have been undertaken. I am also grateful to Dr J. C. Broom for his help in the statistical analysis of my results.

REFERENCES

Balfour, A. (1906). Trypanosomiasis in the Anglo-Egyptian Sudan. *Rep. Wellcome Trop. Res. Lab.* **2**, 113.

Balfour, A. [1909]. Trypanosomiasis in the Anglo-Egyptian Sudan. *Rep. Wellcome Trop. Res. Lab.* **3**, 1908, 27.

Bevan, L. E. W. (1910*a*). Interim report on the animal trypanosomiases of Southern Rhodesia. *Rhod. Agric. J.* **7**, 1029.

Bevan, L. E. W. (1910*b*). Notes concerning *Trypanosoma dimorphon*, with a few preliminary observations on the trypanosomiases of Southern Rhodesia. *Vet. J.* **17** (N.S.), 12.

Blacklock, B. (1912*a*). The trypanosomes found in a horse naturally infected in the Gambia: a double infection. *Ann. Trop. Med. Parasit.* **6**, 107.

Blacklock, B. (1912*b*). The measurements of a thousand examples of a short form of trypanosome from a double infection. *Ann. Trop. Med. Parasit.* **6**, 287.

Broden, A. (1904). Les infections à trypanosomes au Congo chez l'homme et les animaux. *Bull. Soc. Études colon.*, Brussels, February issue.

Bruce, D. (1914). Classification of the African trypanosomes pathogenic to man and domestic animals. *Trans. Soc. Trop. Med. Hyg.* **8**, 1.

Bruce, D., Hamerton, A. E. & Bateman, H. R. (1909). A trypanosome from Zanzibar. *Proc. Roy. Soc.* B, **81**, 14.

Bruce, D., Hamerton, A. E., Bateman, H. R. & Mackie, F. P. (1910*a*). Trypanosome diseases of domestic animals in Uganda. I. *Trypanosoma pecorum*, sp.nov. *Proc. Roy. Soc.* B, **82**, 468.

Bruce, D., Hamerton, A. E., Bateman, H. R., Mackie, F. P. & Bruce, M. E. (1910*b*). Sleeping sickness and other diseases of man and animals in Uganda during the years 1908–9–10. *Trypanosoma brucei*. *Proc. Roy. Soc.* B, **83**, 1.

Bruce, D., Harvey, D., Hamerton, A. E. & Bruce, M. E. (1913). Trypanosome diseases of domestic animals in Nyasaland. III. *Trypanosoma pecorum*. *Proc. Roy. Soc.* B, **87**, 1.

Culwick, A. T. & Fairbairn, H. (1947). Polymorphism in *Trypanosoma simiae* and the morphology of the metacyclic forms. *Trans. R. Soc. Trop. Med. Hyg.* **41**, 415.

Curasson, G. (1943). *Traité de protozoologie vétérinaire et comparée*. I. Trypanosomes. Paris.

Curson, H. H. (1928). Nagana in Zululand. *13th & 14th Reps. Vet. Res. S. Afr.* **1**, 309.

Delanoë, P. (1914). Des variations du pouvoir infectieux et de la virulence de *Trypan. dimorphon* L. et M. (suite). Deuxième note. *Bull. Soc. Pat. exot.* **7**, 58.

Donizio, G. di (1921). Su due tripanosomi del bestiame nella Somalia Italiana. *Bull. Soc. Pat. exot.* **14**, 423.

Dutton, J. E. & Todd, J. L. (1903). First report of the Trypanosomiasis Expedition to Senegambia (1902) of the Liverpool School of Tropical Medicine and Medical Parasitology. *Mem. Lpool Sch. trop. Med.* **11**.

Dutton, J. E., Todd, J. L. & Kinghorn, A. (1907). Cattle trypanosomiasis in the Congo Free State. *Ann. Trop. Med. Parasit.* **1**, 233.

Edington, A. (1908). Preliminary note on the occurrence of a new variety of trypanosomiasis on the Island of Zanzibar. *Proc. Roy. Soc.* B, **80**, 545.

Frizzell, D. L. (1933). Terminology of types. *Amer. Midl. Nat.* **14**, 637.

Fry, W. B. (1911). Animal trypanosomiases in the Anglo-Egyptian Sudan. *Rep. Wellcome Trop. Res. Lab.* **4**, 41.

GODFREY, D. G. (1958). Variations in pathogenicity amongst *congolense*-like trypanosomes in relation to their morphology. *7th Meeting Int. Sci. Committee for Trypanosomiasis Res., Brussels* (in the Press).

HOARE, C. A. (1936*a*). Morphological and taxonomic studies on mammalian trypanosomes. III. *Trypanosoma simiae* and acute porcine trypanosomiasis in tropical Africa. *Trans. R. Soc. Trop. Med. Hyg.* **29**, 619.

HOARE, C. A. (1936*b*). Note on *Trypanosoma simiae* from an outbreak amongst pigs in the Gold Coast. *Trans. R. Soc. Trop. Med. Hyg.* **30**, 315.

HOARE, C. A. (1956). Morphological and taxonomic studies on mammalian trypanosomes. VIII. Revision of *Trypanosoma evansi*. *Parasitology*, **46**, 130.

HOARE, C. A. (1957). The classification of trypanosomes of veterinary and medical importance. *Vet. Rev. Annot.* **3**, 1.

HOARE, C. A. (1958). Revision of the classification of African pathogenic trypanosomes. *6th Meeting Int. Sci. Committee for Trypanosomiasis Res., Salisbury*, 1956 (publ. by C.C.T.A., Hertford), p. 67.

HOARE, C. A. & BROOM, J. C. (1938). Morphological and taxonomic studies on mammalian trypanosomes. IV. Biometrical study of the relationship between *Trypanosoma uniforme* and *T. vivax*. *Trans. R. Soc. Trop. Med. Hyg.* **31**, 517.

HOOF, L. VAN (1924). Action du 'Bayer 205' sur les trypanosomes animales. (Note complémentaire.) *Ann. Soc. belge Méd. trop.* **4**, 255.

HOOF, L. VAN & HENRARD, C. (1933). Recherches sur les trypanosomes pathogènes du bétail à Leopoldville (Congo Belge). *Ann. Soc. belge Méd. trop.* **13**, 495.

HOOF, L. VAN, HENRARD, C. & PEEL, E. (1948). Quelques observations sur les trypanosomiases des grands mammifères au Congo Belge. *Acta trop.* **5**, 327.

JOWETT, W. (1910). Note on a cattle trypanosomiasis of Portuguese East Africa. *J. Comp. Path.* **23**, 251.

KINGHORN, A., YORKE, W. & LLOYD, L. (1913). Final report of the Luangwa Sleeping Sickness Commission of the British East Africa Company 1911–1912. *Ann. Trop. Med. Parasit.* **7**, 184.

KOPKE, A. (1908). Maladie du sommeil et autres trypanosomiases. *Med. Contemporanea* [review in *Bull. Sleep. Sickn. Bur.* **1**, 1909, 241].

LAVERAN, A. & MESNIL, F. (1904*a*). Sur un trypanosome d'Afrique pathogène pour les Equidés, *Tr. dimorphon* Dutton et Todd. *C.R. Acad. Sci., Paris*, **138**, 732.

LAVERAN, A. & MESNIL, F. (1904*b*). *Trypanosomes et trypanosomiases* (1st ed.). Paris.

LAVERAN, A. & MESNIL, F. (1912). *Trypanosomes et trypanosomiases* (2nd ed.). Paris.

LÜHE, M. (1906). Die im Blute schmarotzenden Protozoen und ihre nächsten Verwandten. In Mense's *Handb. Tropenkrankh.* **3**, 69.

MARTIN, G. (1906). *Les trypanosomiases de la Guinée Française*. Paris.

MARTIN, G., LEBOEUF, G. & ROUBAUD, E. (1909). *Rapport de la mission d'études de la maladie du sommeil au Congo Français*, 1906–1908. (Société de Géographie.) Paris.

MARTOGLIO, F. (1911). La peste bovine e le tripanosomiasi nella Somalia Italiana. *Ann. Igiene* (*sper.*) **21** (N.S.), 453.

MENDES, A. C., MORA, A. D., MONTEIRO, A. S. & BRUTO DA COSTA (1909). La maladie du sommeil à l'Ile du Prince. *Arch. Hyg. Pat. exot.* **2**, 271.

MESNIL, F. (1909). [In a letter to Editor.] *Bull. Sleep. Sickn. Bur.* **1**, 435.

MONTGOMERY, R. E. & KINGHORN, A. (1908). A report on trypanosomiasis of domestic stock in North-Western Rhodesia. *Ann. Trop. Med. Parasit.* **2**, 97.

MONTGOMERY, R. E. & KINGHORN, A. (1909*a*). Concerning *Trypanosoma dimorphon* Dutton and Todd: *T. confusum*, sp.nova. *Lancet*, ii, 927.

MONTGOMERY, R. E. & KINGHORN, A. (1909*b*). A further report on trypanosomiasis of domestic stock in Northern Rhodesia (North-Eastern Rhodesia). *Ann. Trop. Med. Parasit.* **3**, 311.

SCHWETZ, J. (1931). Notes morphologiques sur les trypanosomoses animales de Stanleyville et du Congo Oriental. *Ann. Parasit. hum. comp.* **9**, 392.

SCHWETZ, J. (1934). Contribution à l'étude des trypanosomes pathogènes des suidés. (Deuxième étude.) *Bull. agric. Congo belge*, **25**, 313.

SCHWETZ, J. (1938). Quelques réflexions et suggestions pour une future classification des trypanosomes pathogènes de l'Afrique centrale. *Ann. Parasit. hum. comp.* **16**, 265.

SCHWETZ, J. & FORNARA, L. (1929). Y a-t-il des formes d'évolution des trypanosomes chez l'hôte vertébré? *Bull. Soc. Pat. exot.* **22**, 862.

THOMAS, H. W. & BREINL, A. (1905). Trypanosomes, trypanosomiasis and 'Sleeping sickness'. *Mem. Lpool Sch. Trop. Med.* **16**.

TREVAN, J. W. (1956). Statistical analysis of measurements of *Trypanosoma evansi*. (Appendix in HOARE, C. A. (1956), *q.v.*)

YORKE, W. (1914). (Discussion of BRUCE, D. (1914), *q.v.*)

YORKE, W. & BLACKLOCK, B. (1911). The trypanosomes found in two horses naturally infected in the Gambia. *Ann. Trop. Med. Parasit.* **5**, 413.

ON THE BEHAVIOUR OF PIGEON LOUSE, *COLUMBICOLA COLUMBAE* LINN. (MALLOPHAGA)

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(With 6 figures in the Text)

INTRODUCTION

From the literature available it seems that no study has been made of the behaviour of the pigeon louse, *Columbicola columbae* Linn., nor of that of any other Mallophaga. The behaviour of the human louse, *Pediculus*, however, has been studied in great detail by Hase (1915, 1931), Bacot (1917), Nuttall (1917, 1919), Alessandrini (1919), Martini (1918), Pick (1926), Homp (1938), Buxton (1939) and Wigglesworth (1941); while Weber (1929) has given a detailed account of the behaviour of the pig louse, *Haematopinus*. The present paper describes the reactions, under laboratory conditions, of the pigeon louse to three environmental factors, namely temperature, smell and contact. The classification of the mechanism of orientation given by Gunn, Kennedy and Pielou (1937) has been followed here.

GENERAL METHODS AND MATERIAL

The pigeon louse lives normally concealed on the surface of the vanes of feathers. It spends the greater part of its life at rest. The well-fed adult lice of both sexes were collected directly from the pigeon either at the time of performing the experiments or earlier and they were kept in a big Petri dish on the pigeon feathers. There was little difference in the behaviour of lice collected before or at the time of the experiment, but if they were collected more than a week before the experiment they became sluggish and their behaviour was not normal.

The method of experiment and the apparatus used were substantially the same as those described by Wigglesworth (1941). The metal containers used were rectangular, measuring $6 \times 9 \times 12$ cm., the uppermost wall 6×9 cm. The two containers were separated by an asbestos sheet 2 mm. thick, and held together by means of an outer rubber band. The temperature of the containers could be changed to the desired degree by adding ice or by heating water inside them. The temperature was measured by a sensitive resistance thermometer. The arena was circular, glass-walled and 5 cm. in diameter. The floor was usually of paper, except in those cases in which the behaviour was studied on cloth which was stretched on a wooden embroidery frame. Except in temperature experiments the arena rested on a table and the temperature of the surroundings varied between 31 and 32 °C. In temperature experiments half of the arena rested on one container and half on another. The arena was always near the window and the experiments were performed in diffuse daylight in the morning hours.

The reactions of the louse were observed in the arena which was so arranged that

one half differed from the other half in a single factor at a time. A single fresh louse was used for each test. The duration of each experiment varied from 10 to 20 min. Over this range, the duration of the experiments did not seem to have any appreciable influence on the intensity of the reaction. The tracks made by the louse during its movements on the floor of the arena were copied on a sheet of paper by the side of the apparatus, and with the aid of a stop-watch the positions of the louse were marked at 30 sec. intervals. From such tracks it was possible to compare the relative lengths of time spent by the louse in either half of the arena and to determine whether this was due to (i) coming to rest or moving more slowly to one side (orthokinetic reaction), (ii) changing direction more frequently (klinokinetic reaction), or (iii) constantly avoiding one side (klinotactic reaction). When the louse moved against the wall of the arena, its tracks were recorded, for the sake of clearness, as concentric lines.

BEHAVIOUR IN A UNIFORM ARENA

Before interpreting the behaviour under a particular condition it was very essential to have knowledge of the movements of the louse in a uniform arena. It might be mentioned here that there were many individual variations. In some cases the louse would come to rest after moving a minute or two and would continue in this position for some time; in other cases it would go on moving in spirals and so on.

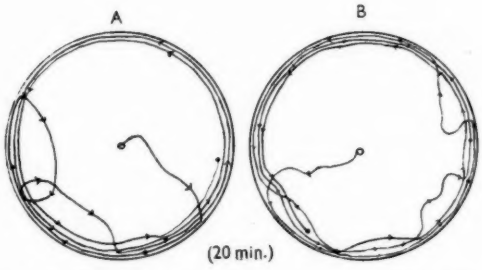

Fig. 1. Tracks followed by the louse in a uniform arena.

But generally the louse moved forward near the wall of the arena often touching the wall with its antenna (Fig. 1). Occasionally the louse crossed the arena at some distance from the wall or turned and walked back. Records were made only of the tracks of individuals showing normal behaviour, which was determined by performing a number of experiments with different individuals under each of the conditions; when a number of individuals showed a more or less similar behaviour, this was taken to be normal.

REACTIONS TO TEMPERATURE

When the louse was given a choice between 23 and 30·5° C. (Fig. 2A), it spent nearly all its time on the warmer side and strongly avoided the cooler side. When it was introduced on the warmer side it remained there and did not enter into the

cooler side. But, on introducing it on the cooler side, it crossed the cooler side as soon as it could and entered into the warmer side, where it remained all the time. The track followed was not straight, but a little convoluted. More or less similar behaviour was observed when the alternative temperatures were 25 and 30·5° C. (Fig. 2B).

Fig. 2C shows a typical track of a louse given a choice between two extreme alternative temperatures 27·5 and 47·5° C. The louse on the warmer side moved very fast and reached the asbestos sheet, but did not enter the cooler side. It continued moving in the middle zone, taking a very irregular convoluted course. It avoided both the temperatures strongly and showed a great preference for the

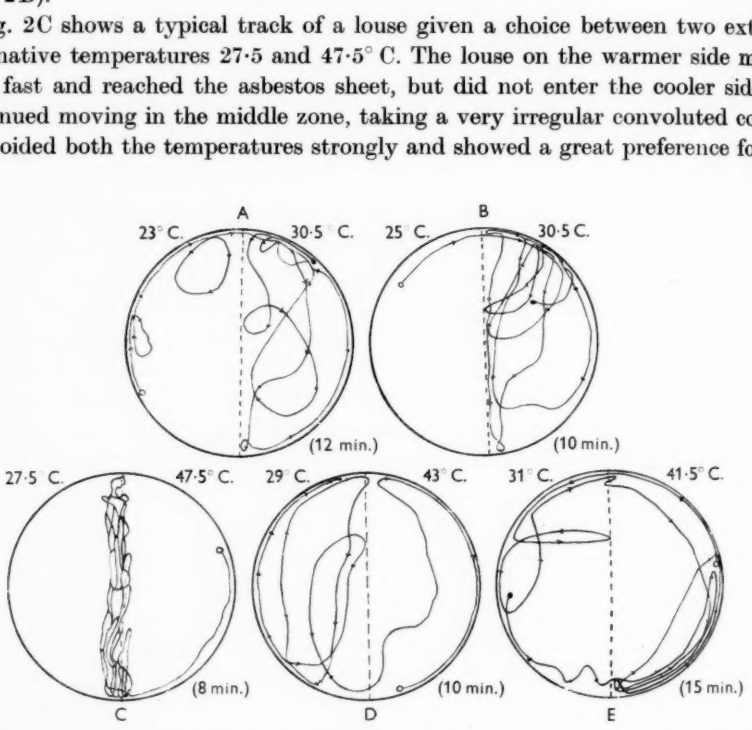

Fig. 2. Tracks of the louse when the two halves of the arena had different temperatures.

middle zone. While it was moving on the asbestos sheet, it touched sometimes the cooler side and sometimes the warmer side. This greatly confused the louse, making its course all the more erratic and the result was a very irregular convoluted track.

When these results were obtained, the response to other differences on the temperature scale was studied in order to find out how sensitive the louse was and its preferred temperatures (thermopreferendum). For this purpose the temperatures were progressively lowered on the warmer side and raised on the cooler side. The temperature differences used varied from 4 to 20° C.

Between two alternative temperatures, 29 and 43° C., the behaviour of the louse became very different. As long as it remained on the warmer side it moved very fast (Fig. 2D) and, after reaching the asbestos sheet, entered the cooler side and spent practically all the time there. This showed that the louse preferred 29° C. in comparison to 43° C., which was strongly avoided. The behaviour of the louse

on the cooler side further suggested that this side was also not liked much, as it often moved towards the centre to find a more suitable place.

When the choice was between 31 and 41·5° C., a very interesting behaviour was shown (Fig. 2E). Instead of avoiding the warmer side, the louse preferred it and avoided the cooler side. However, the avoidance was not so strong as it was when higher temperatures were used. This observation further suggested that the preference to 29° C. in comparison to 43° C. (Fig. 2D) was under compulsion, as the louse had strongly avoided 43° C., so that 29° C. should not be regarded as the thermopreferendum. Again, the louse preferred 36° C. in comparison to 31° C. (Fig. 3A). This behaviour suggested that the thermopreferendum was somewhere between 31 and 41·5° C.

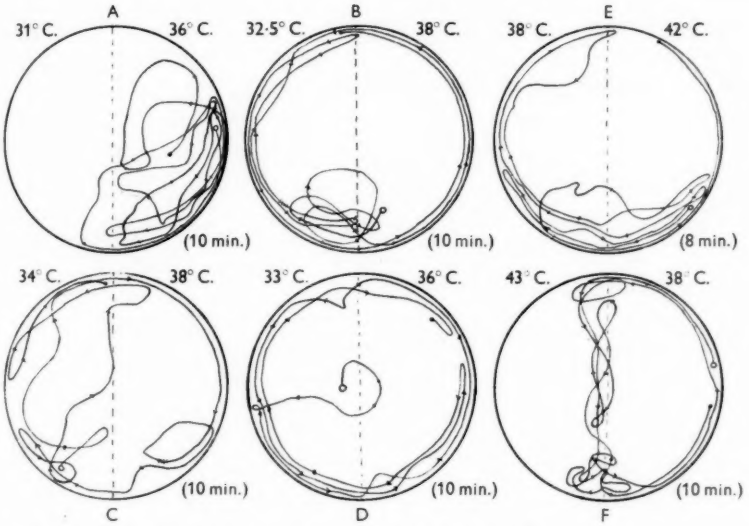

Fig. 3. Tracks of the louse when the two halves of the arena had different temperatures.

To find out the minimum range of the thermopreferendum the choice was given between 32·5 and 38° C. (Fig. 3B). The louse preferred 32·5° C. more, but 38° C. was also not avoided. Similarly, when 34° and 38° C. were used (Fig. 3C), 34° C. was preferred, but the louse could not discriminate between 33 and 36° C. (Fig. 3D). As there was a stronger preference for 34° C. than for 38° C. and the louse could not discriminate between 33 and 36° C., the thermopreferendum was between 33 and 36° C.

When the choice was between 38 and 42° C. (Fig. 3E), 38° C. was preferred, although 42° C. was also not strongly avoided; but, when the temperature was raised to 43° C. on the 42° C. side (Fig. 3F), the raised temperature was strongly avoided, showing that at this range the louse was sensitive to a change of even one degree of temperature.

The results obtained showed that temperatures above 42° and below 30° C. were strongly avoided by the louse. 30–32° and 38–42° C. temperatures were well

tolerated but never preferred to temperatures below 38° and above 32·5° C. The preferred temperature was 33–36° C., and there was no appreciable change in the reaction of the louse to temperature differences within this range. The louse was sensitive to temperature differences of even one degree below and above the range of tolerated temperatures, two degrees difference within the range of tolerated temperatures, and three degrees difference within the range of preferred temperatures.

The tracks of the louse were much convoluted within the range of temperatures strongly avoided. Within this range the louse seemed confused and hesitant and frequently raised its head. When a louse moving on the warmer side reached the cooler side, its speed was lowered. Similarly, when it moved to the warmer side from the cooler zone, the speed increased. The maximum speed was noted at 47° C., when it covered a distance of 50 mm. in 20 sec. (150 mm. per min.). The louse did not show any signs of sensory adaptation to these adverse temperatures, because the speed remained more or less the same throughout under similar conditions.

Mechanism of orientation

The foregoing experiments demonstrated that the louse avoids both high and low temperatures, and prefers moderate temperatures. It appears that at least three mechanisms of orientation contribute to this behaviour. These mechanisms are orthokinesis, klinokinesis, and klinotaxis.

When a louse is exposed to a pair of low and high temperatures in the alternative chamber, and crosses from the low to the high temperature zone, its rate of movement instantly increases. Similarly, when the louse crosses from the high to the low temperature zone, its rate of movement instantly decreases. This simple mechanism of behaviour may be termed orthokinesis.

When it passes from one zone to another the louse often does not follow a straight course, but frequently turns and changes its direction. This mechanism of orientation is known as klinokinesis.

Another mechanism seems to be at work when the two alternative temperatures are cold and hot. None of these temperatures are liked by the louse. Sometimes it moves to one side and sometimes to the other, but it avoids both the zones and remains in the middle part of the arena. This type of mechanism is a directed one and is termed klinotaxis.

It appears that all the three mechanisms are responsible for the general temperature behaviour. But it is difficult to define the exact part played by each mechanism, except in some cases. When there is the sharp avoidance of any one temperature, it may be said that it is due to klinotaxis, as in the case of high and low temperatures. This mechanism is effective in keeping the louse away from unsuitable temperatures. The increased frequency of turning may be regarded as being due to klinokinesis, the mechanism which is important in bringing the louse to preferred temperature. The change in the rate of movement appears to be due to orthokinesis, which, together with klinokinesis, may also be important in avoiding unsuitable temperatures.

REACTIONS TO SMELL

The method used for testing the reactions of the louse towards smell was roughly the same as that used in the temperature experiments. One half of the arena was so arranged as to have the smell being tested.

When one side of the arena was of untreated paper and the other of paper rubbed on a pigeon, the louse strongly avoided the neutral side. The avoidance was so strong that the louse walked almost immediately from the neutral side to the side smelling of pigeon and spent practically all the time there (Fig. 4A).

In another experiment the bottom of the arena was that of voile, and half of it had pigeon feathers underneath. The louse showed definite preference (Fig. 4B) for the side that had the pigeon feathers. The track was much convoluted, perhaps because of some confusion regarding the regularity of the smell.

When one half was neutral and the other contained paper smelling mildly of kerosene, the louse very strongly avoided the side smelling of kerosene (Fig. 4C).

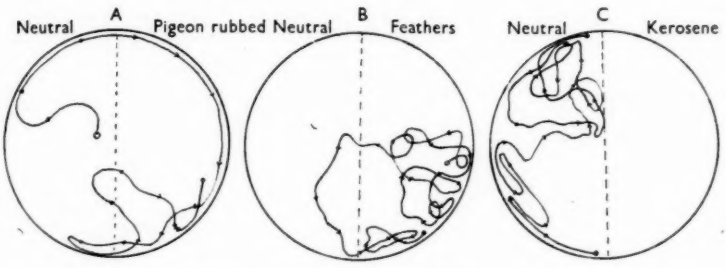

Fig. 4. A and B, reactions of the louse to the smell of pigeon feathers; C, reactions of the louse to the smell of kerosene paper.

Mechanism of orientation

Three mechanisms appear to be involved in orientation of the louse in relation to smell. When the louse enters the favourable side from the unfavourable side, it does not walk straight, but follows a convoluted course, although it definitely avoids the unfavourable side. Klinokinesis is responsible for avoidance of the unfavourable side and for a longer stay on the favourable side. The klinotactic mechanism keeps the louse away from the unfavourable conditions. When it is on the unfavourable side, the louse walks faster and thus is able quickly to avoid unfavourable conditions; by slowing down its movement on the favourable side, it is able to keep longer under favourable conditions. This behaviour is due to orthokinesis.

REACTIONS TO CONTACT

The same type of arena was used as before, each half of the bottom having a different surface. The materials used to make these surfaces were unglazed paper, glazed paper, blotting paper, tin foil, voile, silk and wool.

The louse could not hold on to glazed paper, which was avoided altogether.

When it was left on the glazed paper, it did not move. When it was forced to move, it had to struggle hard, its claws slipping over the surface (Fig. 5A).

When the louse was given a choice between unglazed paper and blotting paper, it preferred the unglazed paper (Fig. 5B) and avoided the blotting paper.

On tin foil the louse experienced more or less the same difficulty as on the glazed paper and preferred even blotting paper to tin foil (Fig. 5C).

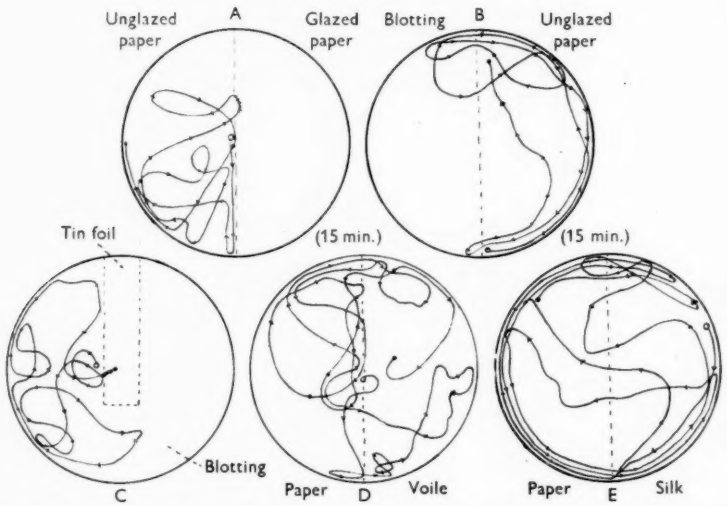

Fig. 5. Reactions of the louse to contact when the two halves of the arena had different surfaces.

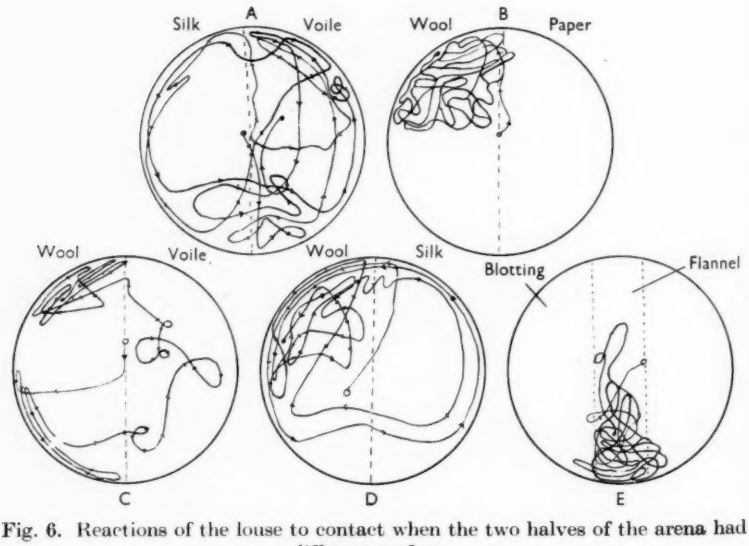

Fig. 6. Reactions of the louse to contact when the two halves of the arena had different surfaces.

When the choice was between voile and unglazed paper (Fig. 5D) or paper and silk (Fig. 5E), the louse preferred the paper, but there was no avoiding reaction towards voile or silk. The louse did not show much discrimination between silk and voile (Fig. 6A).

When the louse was offered paper and wool, it avoided paper strongly (Fig. 6B). On a woollen surface it took a much convoluted course and, when it happened to reach the paper surface, it returned to the woollen side immediately. In the case of wool and voile (Fig. 6C) or wool and silk (Fig. 6D), the preference was again for the woollen surface. Thus the louse showed a strong avoiding reaction towards paper, silk and voile, when the alternative material was wool. It also preferred a frayed strip of flannel to blotting paper, which was then strongly avoided (Fig. 6E).

Mechanism of orientation

The mechanism of orientation to contact appears to be the same as that to other stimuli. Orthokinesis comes into action when the louse moves on paper, voile and silk, on which it walks more actively than on other surfaces. On a glazed surface the louse is unable to walk and comes to rest; this sort of mechanism may be called as negative orthokinesis. On a woollen surface the louse follows a very convoluted track and the mechanism involved appears to be klinokinesis. The distinct avoidance of silk, paper and voile, when the alternative material is wool, is probably due to klinotaxis.

COMPARISON OF THE BEHAVIOUR OF THE PIGEON LOUSE AND THE HUMAN LOUSE

Wigglesworth (1941) has given a detailed account of the behaviour of the human louse and has critically reviewed the literature on this topic. The human louse and pigeon louse belong to two different Orders, but they show similarities in many respects and a comparison of their behaviour is therefore of interest.

The behaviour of both human and pigeon lice is more or less similar in a uniform arena; they generally move near the wall, often touching it with their antennae.

Both human and pigeon lice show thermo-klinotactic, thermo-klinokinetic and thermo-orthokinetic mechanisms of orientation. The thermopreferendum for the two lice depends on the body temperatures of their respective hosts. For the human louse the recorded thermopreferendum is about 30° C. the human body temperature being about 37° C. Similarly, the thermopreferendum for the pigeon louse is about 35° C., the pigeon body temperature being about 42° C. Thus for both the lice the thermopreferendum is about 7° C. below the body temperature of their respective hosts.

A comparison of the tracks of both the human and pigeon lice at different combinations of two alternative temperatures further reveals that their behaviour is very similar to the temperatures below and above their respective thermo-preferenda. It may be pointed out that the behaviour of the two lice appears to be more or less similar when the temperature difference within their thermo-preferenda is the same.

The rate of movement of both the human and pigeon lice is always greater on the warmer side; and no good evidence of thermoadaptation by either louse has been found.

Both the human and pigeon lice are sensitive to the odour of their respective hosts. The human louse shows a definite preference for cloth worn by man for a few days; and the pigeon louse prefers cloth rubbed on a pigeon to neutral cloth.

Neither the human nor the pigeon louse can hold on to glazed paper. When they are forced to walk on glazed paper they have to struggle hard, their claws slipping over the surface. They show a preference for woollen cloth in comparison with unglazed paper and their tracks are then more or less similar in that they follow a convoluted path. They also prefer woollen cloth to cotton. In the case of cotton and silk the pigeon louse shows a definite preference for voile, while the human louse appears to avoid cotton stockings. Their behaviour on frayed flannel is also similar.

SUMMARY

The reactions of the pigeon louse *Columbicola columbae* to temperature, smell and contact have been tested in a circular, glass-walled arena divided into two halves. The thermopreferendum has been found to be between 33 and 36° C., and higher temperatures are more strongly avoided than lower ones. The louse prefers cloth that has been in contact with pigeon feathers to clean cloth. Kerosene serves as a repellant. When the louse is offered different surfaces, namely, glazed paper, tin foil, blotting paper, unglazed paper, voile, silk or wool, the woollen surface is preferred most.

Three mechanisms of orientation, klinotaxis, klinokinesis and orthokinesis appear to contribute to the normal behaviour of the louse. Under certain conditions negative orthokinesis also affects the behaviour.

Comparison of the behaviour of the pigeon louse and human louse has shown that in many respects their behaviour is similar and that their thermopreferendum depends on the body temperatures of their respective hosts.

REFERENCES

ALESSANDRINI, G. (1919). I pidocchi nella profilassi del tifo esantematico. *Ann. Igiene (sper.)*, **29**, 557–98.

BACOT, A. (1917). A contribution to the bionomics of *Pediculus humanus* (*vestimenti*) and *Pediculus capitis*. *Parasitology*, **9**, 228–258.

BUXTON, P. A. (1939). *The Louse*. London: Arnold and Co.

GUNN, D. L., KENNEDY, J. S. & PIELOU, D. P. (1937). Classification of taxes and kinesis. *Nature, Lond.*, **140**, 1064.

HASE, A. (1915). Beiträge zu einer Biologie der Kleiderlaus (*Pediculus corporis* de Geer *vestimenti* Nitzsch.). *Z. angew. Ent.* **2**, 265–359.

HASE, A. (1931). Siphunculata. *Biol. Tiere Dtschl.* **34**, 1–58.

HOMP, R. (1938). Warmeorientierung von *Pediculus vestimenti*. *Z. vergl. Physiol.* **26**, 1–34.

MARTINI, E. (1918). Zur Kenntnis des Verhaltens der Lause gegenüber Wärme. *Z. angew. Ent.* **4**, 34–70.

NUTTALL, G. H. F. (1917). The biology of *Pediculus humanus*. *Parasitology*, **10**, 80–185.

NUTTALL, G. H. F. (1919). The biology of *Pediculus humanus*. Supplementary Notes. *Parasitology*, **11**, 201–20.

PICK, W. (1926). Über den Geruchsinn der Lause. *Derm. Wschr.* **83**, 1020–5.

WEBER, H. (1929). Biologische Untersuchungen an der Schweinelaus (*Haematopinus suis* L.) unter besonderer Berücksichtigung der Sinnesphysiologie. *Z. vergl. Physiol.* **9**, 564–612.

WIGGLESWORTH, V. B. (1941). The sensory physiology of the human louse *Pediculus humanus corporis* De Geer (Anopleura). *Parasitology*, **33**, 67–109.

(*MS. received for publication* 14. v. 1958.—Ed.)

A REVISION OF THE GENUS *CYCLOPODIA* (NYCTERIBIIDAE, DIPTERA)

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(With 77 Figures and 3 Maps in the Text)

In 1835 Westwood described *Nycteribia sykesii* and *N. hopei*, and mentioned the possibility of both being male and female of the same species. He also described *N. dubia*. In 1860 Bigot described *N. oxycephala*. In 1863 Kolenati created the genus *Cyclopodia* for *N. sykesii* and *N. hopei*, but did not include *N. dubia* in the new genus. Kolenati did not see any specimens, but based his diagnosis solely on Westwood's figures and very short descriptions. In 1878 Rondani described *C. albertisi* and *N. ferrarii*. In 1884 Karsch described *C. greeffi* and in 1889 de Meijere described *C. horsfieldi*. All these descriptions, except that of *C. horsfieldi*, are incomplete, and the first paper dealing in detail with ten species, of which four were new, is Speiser's monograph of 1901. Since then only three species have been described (*C. inclita* Falcoz, 1927; *C. pembertoni* Scott, 1932; and *C. tenuis* Schuurmans Stekhoven & Hardenberg, 1938). Speiser re-defined the genus. He mentioned the pigmented eyes with two ocelli, the broad antennae, the long anterior coxae and the three rings on the tibiae.

N. roylii Westwood was placed by Scott (1908, 1914) into the genus *Cyclopodia* because of its long tibiae with three rings and the presence of eyes with two ocelli. Scott (1917) recognized the marked differences of *N. roylii* from the other species of *Cyclopodia*, mainly in the structure of the head, and created for it the subgenus *Paracyclopodia* of the genus *Cyclopodia*. Theodor (1954) removed the subgenus from *Cyclopodia* and considered it provisionally as a separate genus because the structure of head and eyes, thorax and male genitalia clearly proved that this species is much more closely related to the genus *Basilia*. It was not then placed into the genus *Basilia* because the structure of the female abdomen differs markedly from that of other species of *Basilia*. The finding of the female of *Basilia bouvieri* Falcoz (= *Tripselia* (*Neotripselia*) *bouvieri* Falcoz) proved, however, that this species is transitional between *N. roylii* and other species of *Basilia*, and that the peculiar structure of the female abdomen is apparently a specific development. There seemed therefore to be no reason for maintaining *Paracyclopodia* as a separate genus, and it is now considered as a subgenus of the genus *Basilia* (Theodor, 1957).

Some details of the external genitalia of several species were given by Karaman (1939, 1948) who, however, misinterpreted the phallobase as the aedeagus. The structure of the genitalia was described in detail by Theodor in 1953 and 1954.

A detailed study of the structure of the head of *C. sykesii* has been published by Jobling (1928) and some aspects of its anatomy by Mukerji & Dasgupta (1954).

Genus **Cyclopodia** Kolenati, 1863

Type species: *Cyclopodia sykesii* (Westwood) 1835

Diagnosis. Head compressed dorso-ventrally. Eyes consisting of two more or less deeply separated ocelli. Palps either broad and laterally compressed or slender and tapering. They bear marginal setae and a long or short terminal seta. Antennae with a broad dorsal process of the basal segment. A row of 3 to 8 minute spines at the posterior margin of the theca of the proboscis in each half (Fig. 1).

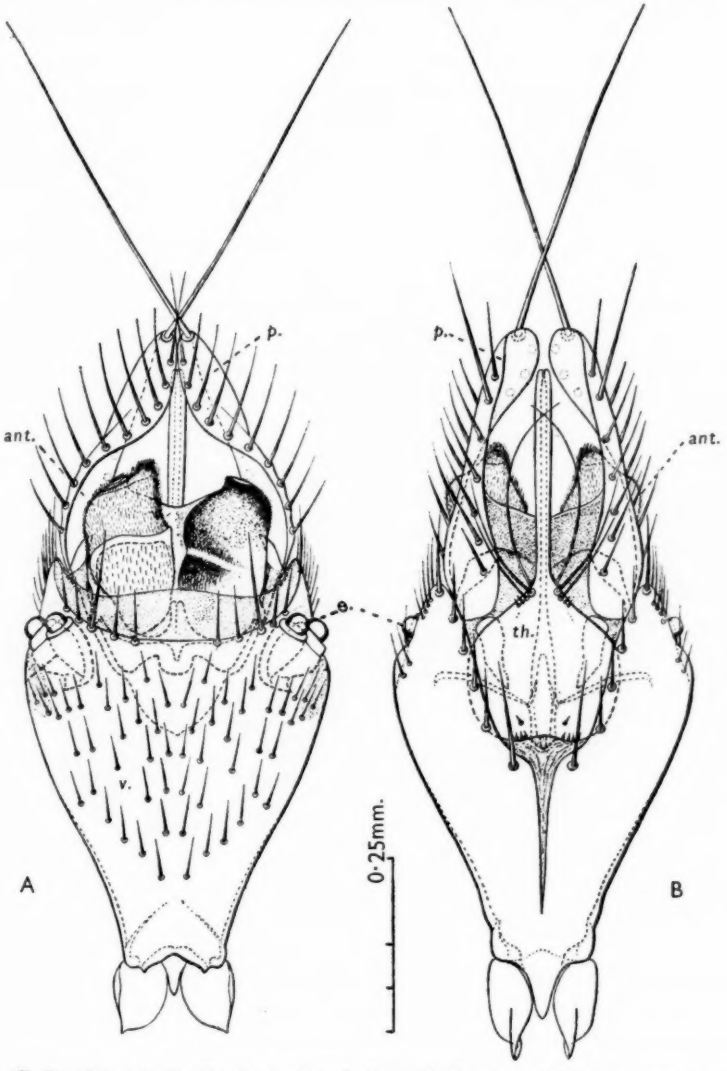


Fig. 1. *Cyclopodia sykesii*. Head: A, dorsal; B, ventral; *ant.*, antenna; *e.*, eye; *p.*, palp; *th.*, theca of labium; *v.*, vertex. (After Jobling, *Parasitology*, **20**, 256, 1928.)

Thorax (Fig. 2, *a*) rounded anteriorly. Thoracic ctenidia with thick, blunt teeth. Notopleural sutures diverging posteriorly. Notopleural setae 1 to 4 in a row in most species, in a group of 7–8 setae in one species, altogether absent in another. They stand close to the haltere groove at the base of the mesopleural sutures. Haltere groove either open or partly or completely closed by a covering flap (Fig. 3). Oblique sutures well developed, open, sometimes rounded anteriorly. Coxae 1 very long, club-shaped. Tibiae long, nearly parallel sided, slightly compressed laterally, with 3 rings of weaker integument in the middle (Fig. 2*b*). Some moderately long setae at the basal margin of the rings.

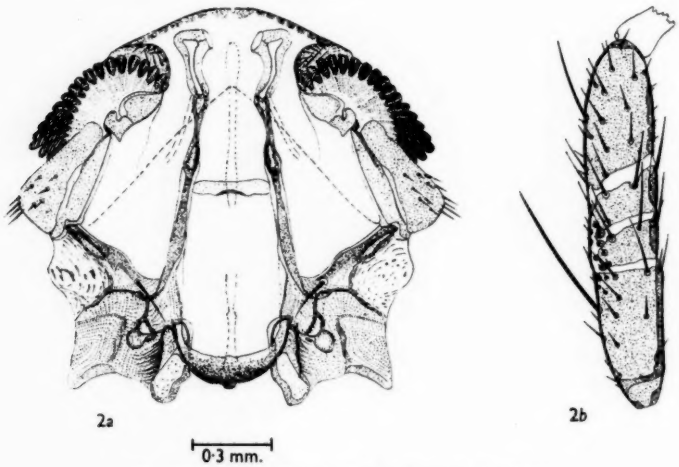


Fig. 2. *Cyclopodia greeffi*. *a*, thorax, *b*, tibia.

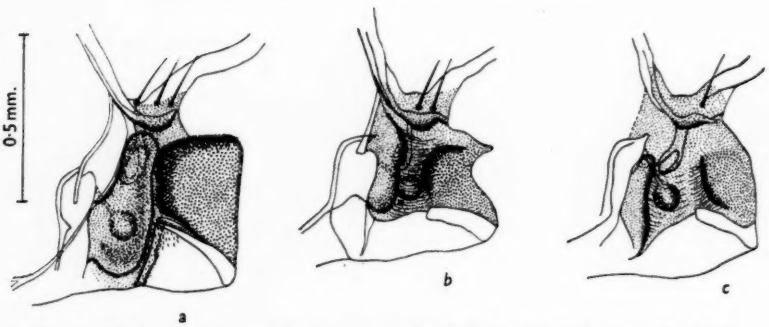


Fig. 3. Haltere groove. *a*, *Cyclopodia sykesii*; *b*, *C. pembertoni*; *c*, *C. greeffi*.

Abdomen. Tergites 1 and 2 fused in both sexes. Postspiracular sclerite without setae in most species. Segmentation in the female much reduced, only the basal and anal sclerite always sclerotized. Abdominal ctenidium consisting of thick, blunt teeth. Sternite 7 normal or transformed into a genital plate which bears spines posteriorly. Abdomen of male with normal segmentation. A row of spines of

varying form on the posterior margin of sternite 5 in one subgenus, absent in the other.

Genitalia. Aedeagus either membranous with a rod-like structure in its wall, without apodeme, completely invaginated inside the abdomen in the resting position, phallobase triangular with apical hook (subgenus *Cyclopodia.* s. str.) or aedeagus partly or completely sclerotized, with apodeme and a phallobase which is bifid in some species (subgenus *Leptocyclopodia* subg.nov.).

Structure of the genitalia. The genitalia of *C. greeffi* have been described in detail previously (Theodor, 1953, 1954, 1957) and some details are added in the present paper. Study of the genitalia of this genus is particularly difficult, as it is nearly impossible to recognize the various parts and their function while they are retracted inside the abdomen, and details can only be made out in the few specimens which have been conserved with the genitalia extended or on living material. The following description is thus based on the genitalia of *C. greeffi* and *C. sykesii*, mainly because sufficient material of these two species was available, including a number of specimens with their genitalia extended (Fig. 4).

The aedeagus is a tapering membranous tube with a sclerotized ridge in its wall. The ductus ejaculatorius runs through the aedeagus, opening near its tip, sometimes in a specially differentiated part which may be articulated with the aedeagus rod. The aedeagus continues basally in a membranous tube, the connecting tube, which bears a field of spines at its ventral surface. These spines are directed backwards when the genitalia are extended. The connecting tube may widen into a bulb near its base which also bears spines. The form and length of the aedeagus rod and of its apical part, as well as the extent of the field of spines on the connecting tube, differs in the various species. The phallobase is a triangular plate with an apical curved hook which closes the genital opening in the resting position. The phallobase articulates basally with the basal arc which is a broad plate with an anterior process. The basal arc articulates posteriorly with a sclerite with two lateral processes, the basal plate. Basal arc and basal plate form a frame from which the connecting tube is everted. The apical lateral parts of the phallobase are prolonged into two long processes or ridges in the wall of the connecting tube which support it ventrally and laterally. At the distal end of the connecting tube lies a U-shaped sclerite which forms a frame from which the aedeagus turns out. This sclerite articulates with the aedeagus rod. The sclerite has long arms in some species, e.g. *C. sykesii* and short arms in others, *e.g. C. greeffi*. It is absent in others. The parameres are small, triangular, rounded, or elliptical sclerites which close the genital opening at the apical ventral part of the phallobase.

In the resting position the connecting tube is invaginated inside the abdomen and the aedeagus lies inside it. The aedeagus and the connecting tube are everted by contraction of the abdomen and the resulting pressure of the body fluid. Retraction is effected by a muscle which originates on sternite 5 and which is inserted at the base of the aedeagus. This muscle consists of two parts which originate separately at the sides of the sternite. The muscle is formed by spirally arranged fibres, as in a rope, which cause the muscle to twist into a knot on contraction. The aedeagus inside the connecting tube lies further basally in the resting position

than the points of origin of the muscle, and it has to be assumed that the aedeagus is either pulled into its position by negative pressure when the contraction of the abdomen relaxes or is pushed into position by contraction of the muscles of the anal segment. In the everted position the muscle forms a very long strip which runs from the sternite around the articulation of the phallobase with the basal arc through the connecting tube to the base of the aedeagus. The ductus ejaculatorius, which opens into the base of the aedeagus, is pulled along into the tube with the muscle.

In *C. ferrarii*, *C. macrura* and other related species there is a type of genitalia which resembles in many respects that of *Eucampsipoda*. There is a more or less sclerotized aedeagus with a long apodeme. Phallobase and basal arc are either fused or articulated. The phallobase has in some species an armature of spines on the surface and may be bifid. In *C. macrura* and some other species the aedeagus

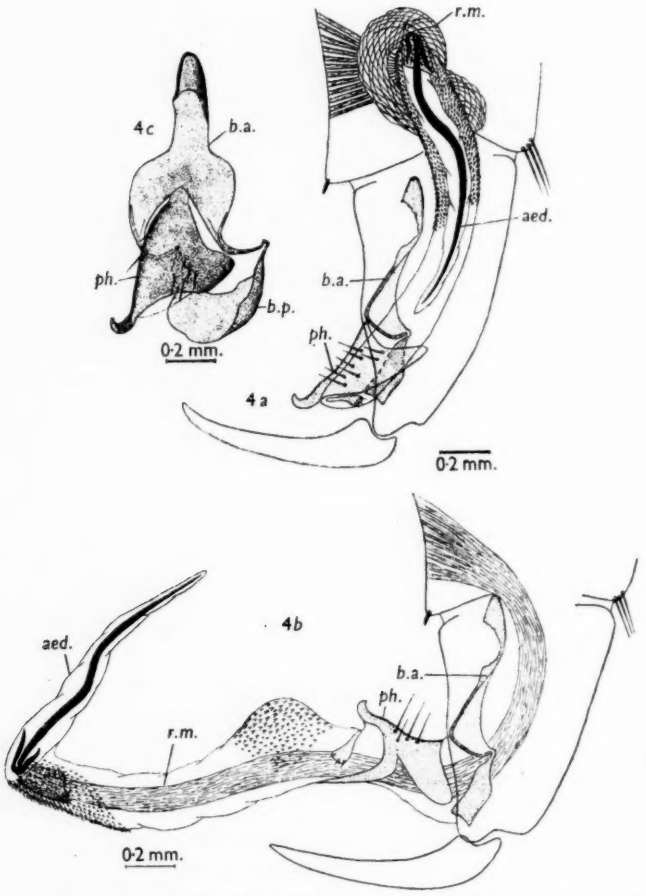


Fig. 4. *Cyclopodia greeffi*. Male genitalia. *a*, contracted; *b*, extended; *c*, basal arc, phallobase, and basal plate; *aed.*, aedeagus; *b.a.*, basal arc; *b.p.*, basal plate; *r.m.*, retractor muscle; *ph.*, phallobase.

is wholly sclerotized. In *C. macrura* it somewhat resembles that of some species of *Basilia*, being curved and tapering to a point. The aedeagus of *C. ferrarii* is membranous in its greater part, and is sclerotized in two lateral arms which are broadening apically and bear a number of backwards pointing teeth ventrally near the end (Fig. 53). The two lateral arms spread laterally when the aedeagus is everted. Its extension is limited by two sclerotized bands, carrying small teeth, which run from the base of the aedeagus to the posterior end of the parameres. The aedeagus is inflated when extended, apparently resembling the endophallus of *Eucampsipoda* in its function. The parameres are recognizable in *C. ferrarii*, where they form the apical ventral part of the phallobase, but they are not mobile as in other genera. In *C. macrura* they are completely fused with the phallobase. The basal plate in these species has become very elongated, with an anterior concavity.

According to the structure of the genitalia, the form of the anal segment and sternite 5 of the male and the segmentation of the abdomen of the female, the genus *Cyclopodia* can be divided into two groups. In *C. ferrarii*, *C. macrura* and other related species the anal segment of the male is very long and slender, the claspers may bear a characteristic armature of spines and the genitalia resemble those of *Eucampsipoda* and *Dipseliopoda* in having a sclerotized aedeagus with a long apodeme. There is, however, no connecting membrane and no endophallus. Tergite 5 of the male is as long as, or longer than, tergite 4 and the row of specialized spines at the hind-margin of sternite 5, which is present in other species of *Cyclopodia*, is absent. These species also resemble *Eucampsipoda* and *Dipseliopoda* in the less reduced segmentation of the abdomen in the female. There is a sclerite on tergite 6 and sclerites on sternites 5–7 or 6 and 7. The posterior margins of sternites and tergites on the membranous part of the abdomen are more or less clearly marked by rows of setae. The postspiracular sclerite bears small spines in some species as in *Eucampsipoda*. These species differ from the other species of the genus so markedly in the characters mentioned that the creation of a separate subgenus for them seems justified, and the name *Leptocyclopodia* subg.nov. is proposed. The other species of the genus have the very much modified genitalia described above for *C. sykesii* and *C. greeffi*. In the female of these species the segmentation of the abdomen is so strongly reduced that the limits of the segments are scarcely recognizable. There are no sclerites between the basal sclerites and the anal segment and sternite 7 is transformed into a genital plate which covers the genital opening from the ventral side. (In the subgenus *Leptocyclopodia* sternite 7 is not so modified.) Tergites 5 and 6 in the male of these species are always much shorter than 3 and 4 and there is always a row of thick, specialized spines on sternite 5. These species will be considered as forming the subgenus *Cyclopodia* s.str.

Subgenus **Cyclopodia** s.str.

Type species: *Cyclopodia sykesii* (Westwood) 1835

Diagnosis. Palps laterally compressed, broad, with short terminal setae. Sclerite connecting the thoracic ctenidium with coxa 2 more or less narrowly triangular and covered with several rows of setae. Tergites of the male abdomen with either

continuous or interrupted marginal rows of setae or short spines. Tergites 5 and 6 always much shorter than the preceding tergites. Anal segment of the male broadly conical, less than twice as long as wide at the base. A row of short thick spines at the hindmargin of sternite 5 of the male. Male genitalia: aedeagus membranous, without apodeme, completely invaginated inside the abdomen in the resting position. There is a connecting tube which bears a field of spines. Segmentation of the abdomen of the female much reduced. No sclerites between the basal sclerites and the anal segment, except sternite 7 which is transformed into a genital plate with spines at the posterior margin. Anal sclerite absent.

The subgenus can be divided into the four following groups:

(1) *sykesii* group. 10 species. Oriental and Pacific Region. Size 4–5·5 mm. Eyes large. Setae on anterior dorsal margin of head and on the vertex present. Haltere groove completely closed by a cover which is attached only in its posterior part on the median side (Fig. 3). Tergites of the male abdomen with marginal rows of setae which are broadly interrupted in the middle. (*C. australis*, which belongs to this group according to all other characters, has a different arrangement of the marginal rows. There are only short spines on tergites 2 and 3, a group of setae in the middle on tergite 4 and continuous rows on tergites 5 and 6.) Spines on sternite 5 club-shaped. Spiracles of female abdomen large (80–100μ) (Fig. 5). Genital plate with thick spines posteriorly.

(2) *pembertoni* group. 2 species. Pacific Region. Size 3·5–4 mm. Eyes small. Tergites of male abdomen with continuous marginal rows. Spines on sternite 5 club-shaped. Haltere groove partly closed by a narrow flap which is attached along its whole length and by a large rounded bulge opposite. Spiracles of female abdomen small (50μ). Vertex either as in *sykesii* group or bare. Genital plate with thick spines posteriorly.

One species (*C. minor*) is intermediate between the *sykesii* and *pembertoni* groups. The eyes are large as in the *sykesii* group. The arrangement of setae on the head differs from that of both groups in having only 2 setae at the anterior margin and some setae on the posterior part of the vertex. The cover of the haltere groove resembles that of the *pembertoni* group in its being attached to the notopleural suture along its whole length, but it is larger, covering the groove nearly completely. The marginal rows of the tergites of the male abdomen are interrupted on tergites 2 and 3 and continuous on tergites 4 to 6.

(3) *greeffi* group. 2 species. Ethiopian Region and Madagascar. Size 4–5 mm. Eyes large. Vertex bare, anterior margin of head with 2 setae or bare. Haltere groove open. Tergites of male abdomen with continuous marginal rows. Spines on sternite 5 not club-shaped. Spiracles of female abdomen small (50μ). Genital plate with thick spines posteriorly.

(4) *tenuis* group. 4 species. Oriental and Pacific Region. Size 2–3 mm. Vertex bare. A few setae at the anterior margin of the head. Haltere groove open. Tergites of the male abdomen with continuous marginal rows. Spines on sternite 5 club-shaped. Abdomen of female without any groups of long setae. Spiracles of female abdomen small (30μ). Genital plate with thin spines posteriorly.

SYKESII GROUP

(1) **Cyclopodia sykesii** (Westwood) 1835)

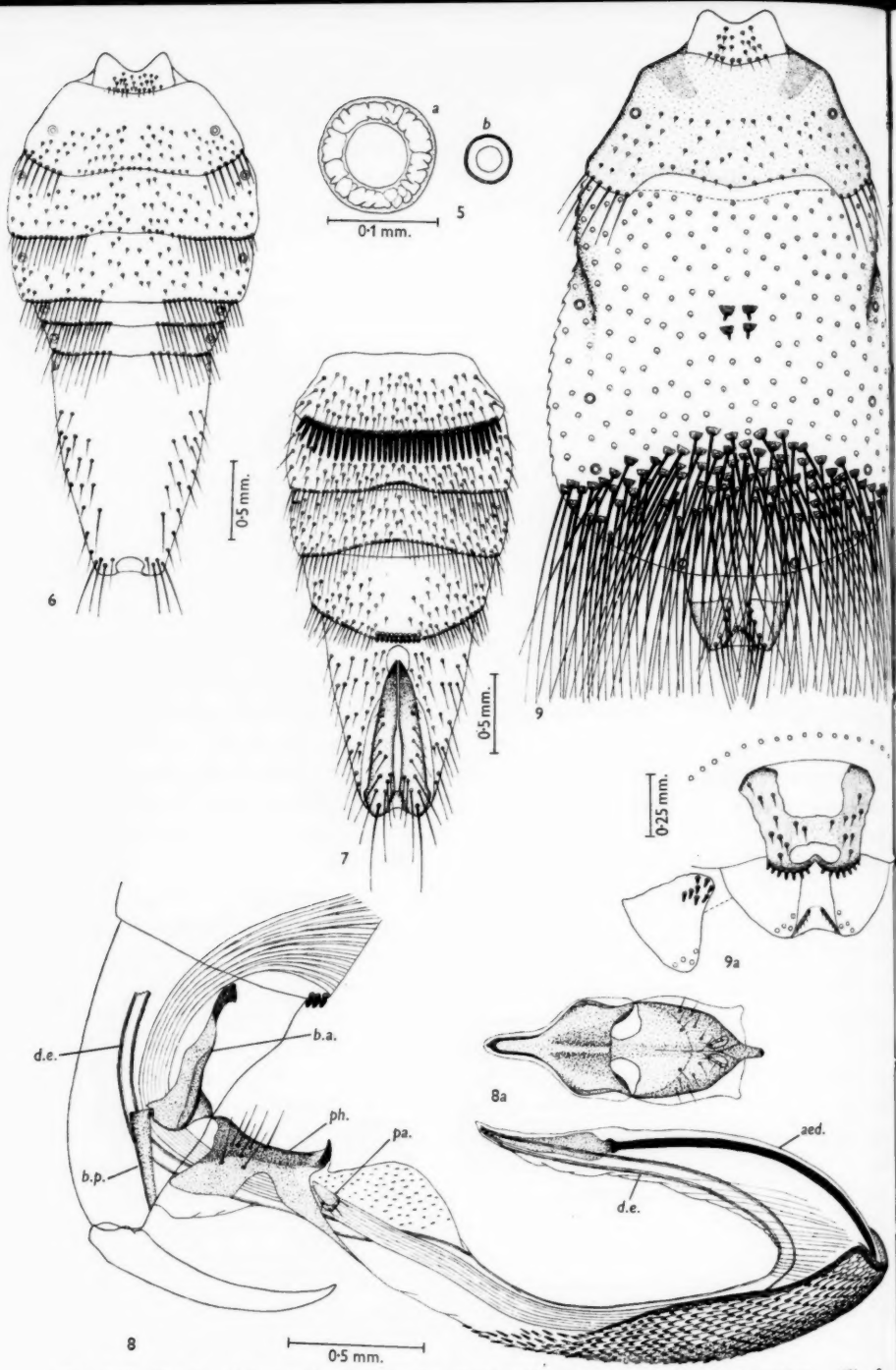
Syn. *Cyclopodia hopei* (Westwood) 1835)

Description. Length 4–5 mm. Colour dark brown.

Head (Fig. 1) dorso-ventrally compressed. Vertex covered with short setae. A row of 6–8 setae at the anterior dorsal margin of the head with a gap in the middle. Eyes large with deeply separated ocelli which have the lenses clearly set off from the base. Palps broad, with a marginal row of short setae and a terminal seta which is about as long as the palp. The marginal setae are longer towards the end of the palp. Labella of labium about 1·5 times as long as the theca. A row of 4–6 minute spines at the posterior margin of the theca in each half.

Thorax (measured at the sternal plate) slightly wider than long, rounded anteriorly. Median suture narrow, indistinct in the middle third. Oblique sutures convex anteriorly near the median suture, forming an angle of slightly less than 90°. Sternal plate covered with short spines except anteriorly, between the ctenidia, a spot behind the posterior end of the oblique sutures and another near the posterior part of the lateral margin. These areas are covered with minute hairs which are also scattered between the spines. The posterior margin of the sternal plate bears a row of moderately long setae laterally and is bare in the middle. Thoracic ctenidia with about 15 thick, blunt, curved teeth with secondary striations. Mesonotum widening posteriorly. Mesopleural sutures narrow, slightly curved, with 3 notopleural setae near the base. Haltere groove covered by a triangular cover attached in its posterior lateral part. Legs long, tibiae cylindrical, slightly compressed laterally, with 3 rings of weaker integument in the basal 3/5 of the tibia. A few thin setae of moderate length at the basal margin of the rings.

Abdomen, male (Figs. 6–7). Tergite 1 + 2 with rounded posterior margin and a row of 5 to 6 moderately long setae at the lateral posterior corners. Posterior part of the surface covered with short spines, some of which are on the otherwise bare posterior margin. Tergite 1 (the membranous area at the base of the sclerite) with a group of 25 to 30 small spines. The anterior rows consist of minute spines, those of the posterior rows are much larger. Tergites 3 and 4 with straight posterior margin and marginal rows of closely standing setae of moderate length in the lateral thirds. The middle part is bare except for a few minute spines. Small spines are scattered over the surface of the tergites except for an anterior bare stripe. Tergites 5 and 6 much shorter than 4, bare on the surface, with marginal rows of longer setae and a narrower gap in the middle. Anal segment broadly conical, with moderately long setae in the lateral posterior parts of the dorsal surface and some long setae posteriorly. Sternite 1 + 2 short, trapezoid, with a ctenidium of about 32 blunt, broad teeth. Surface covered with setae which are very short in the middle anteriorly and longer laterally. Sternites 3 and 4 with slightly concave posterior margin which bears a dense row of setae which are longer laterally. Surface covered with short setae which are longer laterally and posteriorly. Sternite 5 longer, with convex posterior margin which bears a row of about 10 short, barrel-shaped spines in the middle, slightly before the margin and a row of thin



Figs. 5–9. Fig. 5. Abdominal spiracles of *a*, *Cyclopodia sykesii*; *b*, *C. greeffi* (females). Fig. 6. *C. sykesii*: male, abdomen, dorsal. Fig. 7. *C. sykesii*: male, abdomen, ventral. Fig. 8. *C. sykesii*: male, genitalia, *a*, extended; *d.e.*, ductus ejaculatorius; *pa.*, paramere. Other letters as in Fig. 4. Fig. 9. *C. sykesii*: female, abdomen, dorsal. Fig. 9*a*, genital plate.

setae behind them. There are longer setae at the lateral parts of the posterior margin and shorter setae on the lateral posterior parts of the surface. Anal segment with an anterior median bridge ventrally.

Genitalia (Fig. 8). Claspers straight, slender, tapering to a darkly pigmented blunt point, 5–6 times as long as thick at the base. They bear some long setae near the base and some shorter setae up to the middle on the dorsal surface. Some very short spines in the apical part. Basal arc with broad, rounded anterior process, rounded shoulders and concave posterior margin. Phallobase triangular, with a long apical hook and a double row of about 12 short setae across the middle. Basal plate square with concave anterior margin. Parameres small, narrowly elliptical, with 2–3 short setae posteriorly. The aedeagus rod articulates with a leaf-shaped apical part. A long U-shaped ridge in the apical part of the connecting tube which forms a frame around the base of the aedeagus in the extended position. Aedeagus rod 1·3–1·5 mm. long without the apical part. The field of spines of the connecting tube covers its whole ventral surface and is as long as, or slightly longer than, the aedeagus rod.

Abdomen, female (Fig. 9). Ovoid in distended specimens, slightly flattened dorso-ventrally. Spiracles large (80–90μ). Tergite 1 + 2 as in the male, but longer and there are 5–8 setae at the posterior lateral corners which are longer than in the male. The dorsum between tergite 2 and the anal segment is membranous without any sclerites. It is covered in its anterior 2/3 with minute spines with sclerotized bases which stand closer together anteriorly. In the middle of the dorsum is a bare area which bears some large, blunt spines on pigmented sclerotized bases. Most specimens have 4 such spines, arranged in a square, some have 5 or 6 or only 2 or 3 such spines. There are no small spines between the large ones. The posterior third of the dorsum, between spiracles 5 and 6, is covered with about 6 rows of very long and strong setae. These setae are separated from the anterior group of spines by several rows of minute spines. The area behind the setae, in front of the anal segment, is bare. The anal segment is short, conical, sclerotized and bears a row of 6–8 long setae, which become shorter ventrally, parallel to the anal frame on each side. Sternite 1 + 2 as in the male, but longer. Ventral surface of the abdomen covered with short spines which are, however, longer than on the dorsum. They are longest anteriorly in the middle and much shorter in the lateral posterior parts of the venter. The last row consists of short spines laterally and about 10 long setae in the middle opposite the genital plate. This is roughly square, sclerotized laterally, with a bridge connecting both sides, forming an H. It bears a row of 6 to 8 short, thick spines on each of the posterior broadened ends of the H. The surface of the plate is covered with short spines. Two groups of 8 to 10 short spines at the interior ventral corners of the anal sclerites are covered by the genital plate in most specimens. There are no longer setae or bare spaces indicating the margins of segments on the ventral surface of the abdomen.

Distribution and hosts. C. sykesii has been recorded and material has been examined from the whole Indian Peninsula (except from the North West), from Ceylon and the Maldive Islands. Eastwards it has been recorded from N.E. Assam and south to Rangoon. Its distribution in Burma, Siam and South East

China is not known. *Pteropus giganteus* and its subspecies are apparently its main hosts. Records from *P. vampyrus* and other species will have to be checked, as *C. sykesii* has not always been separated in the past from *C. horsfieldi* (see p. 305).

The record from Judaea in Palestine was probably due to a mistake in labelling (Theodor, 1954).

(2) **Cyclopodia horsfieldi** de Meijere, 1899

Syn. (?) *Cyclopodia magna* Kishida, 1932

Ferris (1925) gave figures for specimens from the Philippines. This species resembles *C. sykesii* so closely in the male that single males cannot be identified. No chaetotactic characters or characters in the genitalia have been found which permit the separation of males of the two species.

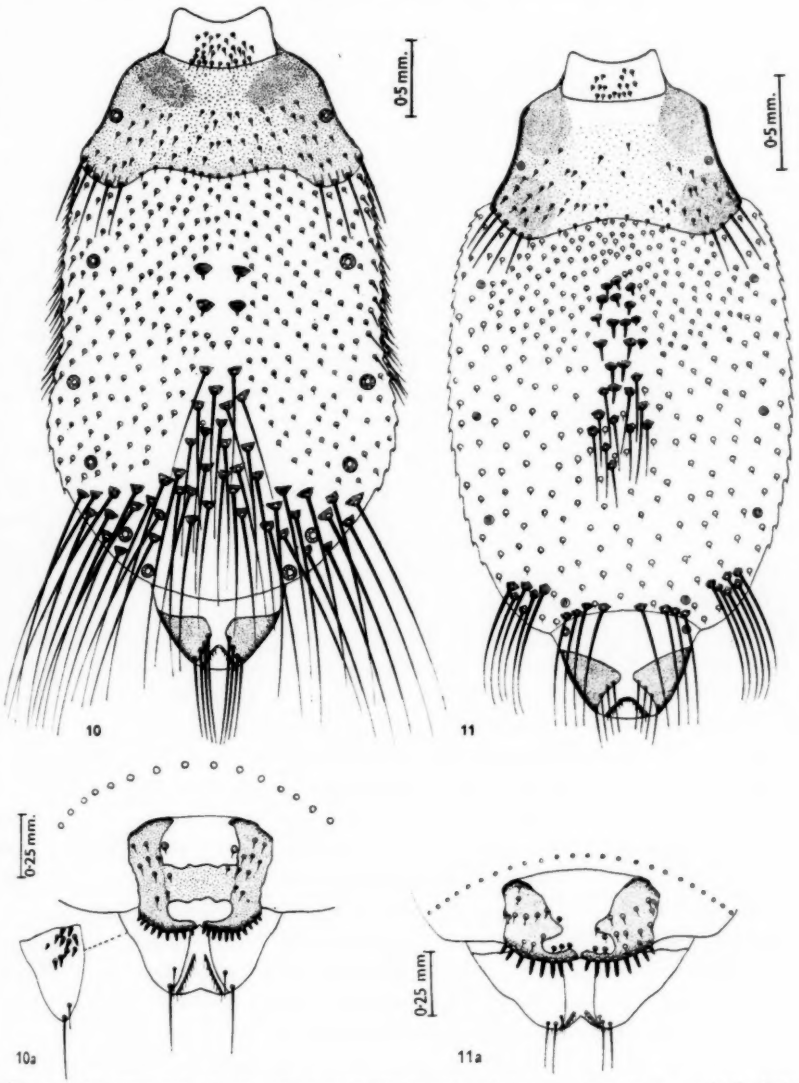
The female differs from that of *C. sykesii* in the chaetotaxy of the abdomen (Fig. 10). The group of long setae on the posterior part of the dorsum consists of only about 3–4 transverse rows of more widely spaced setae which reach laterally further beyond the spiracles than in *C. sykesii*. This group continues anteriorly in a median stripe which reaches the anterior group of large spines in some specimens. In others the anterior field of spines and the group of long setae are separated by a varying number of rows of the minute spines which cover the rest of the dorsum so that some specimens resemble *C. sykesii*. Females of *C. horsfieldi* can, however, always be differentiated from *C. sykesii* by the presence of longer setae on the pleurae. The setae on the anterior part of the pleurae, anterior and ventral of spiracle 4 are much longer than those on the posterior part of the pleurae and on the venter. In some very distended specimens faint indications of segmentation can be made out, e.g. the marginal rows of sternite 5 may consist of slightly longer setae with a narrow bare space behind them. In other equally distended specimens no such indication of segmentation is present. The genital plate is similar to that of *C. sykesii*, but there are about 10 spines in the posterior row in each half. The group of spines at the ventral median corners of the anal sclerites consists of 12–15 spines of varying size. The spiracles are even larger than in *C. sykesii*, 100–110 μ in diameter.

Distribution and hosts. Throughout the Malaysian Region including Celebes and the Philippines. Material examined from the Malay Peninsula, Siam near Bangkok, Sumatra, Java, Borneo, Celebes, Bonerate Island near Flores, Philippines. (See map no. 1, p. 306.) Recorded from *Pteropus vampyrus* and subspecies, and from *P. alecto* (Celebes), *P. hypomelanus* and its subspecies, *P. philippinensis* and *P. speciosus* in the Philippines.

The close similarity of the males of *C. horsfieldi* and *C. sykesii* and the variability of the female of *C. horsfieldi* suggest that *C. horsfieldi* is possibly only a subspecies of *C. sykesii*, replacing it in the Malaysian Region. This, however, can only be decided when more material from Burma and neighbouring countries, where both species may occur together, will be available.

Kishida (1932) described *Cyclopodia magna*. According to the figure of the male this species belongs to the *sykesii* group as it shows interrupted marginal rows of setae on the abdominal tergites. Kishida mentions in the description of the female

that 'the posterior part of the dorsum is covered with many long setae', and 'it occurs on big bats from the south of Japan to the Philippines'. It is thus possibly identical with *C. horsfieldi* which occurs in the Philippines and perhaps further north. The distribution of a number of Oriental species of Nycteribiidae extends into the southern part of the Palaearctic Region in the Far East. Ellerman & Morrison Scott (1951) and Eisentraut (1944) do not mention any species of Pteropidae from Southern Japan proper, but give the Riu Kiu Islands as the most



Figs. 10, 11. Fig. 10. *Cyclopodia horsfieldi*: female, abdomen, dorsal. *a*, genital plate.
Fig. 11. *C. similis*: female, abdomen, dorsal. *a*, genital plate.

northern point of their distribution. Kishida does not give an exact locality for *Cyclopodia magna* but he includes the Riu Kiu Islands and Formosa in the area he calls Southern Japan in his descriptions.

(3) **Cyclopodia similis** Speiser, 1901

Length 5 mm. Colour reddish brown. Head, thorax and legs as in *C. sykesii*.

Abdomen, male also as in *C. sykesii* in general. The minute spines on tergite 1 are only 18 to 22 in number and the posterior spines are not much longer than the anterior ones.

Genitalia. Claspers 4 times as long as broad at the base. Basal arc with sloping shoulders and long anterior process. Phallobase with narrow, long hook and an irregular double row of setae across the base. Parameres narrowly triangular with 2 rather long setae at the end. Aedeagus rod 0·85 mm. long. Field of spines as long as the aedeagus rod (Fig. 77*c*).

Abdomen, female (Fig. 11). Tergite 1 + 2 as in *C. sykesii*, but the number of setae in the lateral posterior corners varies from 2 to 6. Dorsum of abdomen covered with minute spines. In the middle of the dorsum there is an oblong group consisting of 7 to 12 large blunt spines on sclerotized bases anteriorly and 7 to 12 moderately long setae posteriorly. A row of long setae, at the posterior border of the dorsum, between spiracles 6. Two groups of long setae, each consisting of 6–8 setae, in 2–3 rows lateral to spiracle 6. These setae are separated from the dorsal group by a broad space covered with minute spines. In one specimen there are only 3 setae lateral to spiracle 6 and the row across the middle is missing. In this specimen the dorsal group consists of 7 spines and 7 setae. Anal segment as in *C. sykesii*. Sternite 1 + 2 short, with a ctenidium of 38–40 teeth. Ventral surface covered with short spines. In some distended specimens some narrow bare spaces exist between the sternites, best marked between sternites 5 and 6, and there are a few longer setae in the posterior row of sternite 5. There is a row of moderately long setae in the middle of the marginal row of sternite 6, opposite the genital plate. This is square, consisting of two lateral sclerites which are not connected by a bridge as in *C. sykesii*, but separated by a Y-shaped membranous median stripe, the wide part of which is anterior. There are about 8 spines, which are longer than in *C. sykesii*, at the posterior margin in each half. The group of spines at the ventral median corners of the anal sclerites consists of 7–8 spines. The surface of the lateral sclerites of the genital plate is covered with short spines. There is a group of about 12 long setae on the pleurae between spiracles 4 and 5 in some specimens. In one specimen with otherwise normal development of setae there are only 2 such setae and in the above-mentioned specimen, with reduction of the posterior groups, this group is fully developed.

Material examined. New Britain, *Pteropus neohibernicus*, 2 cotypes (Dahl.) Larat. Tanimbar Islands, 5 specimens (Muir). (Specimens discussed by Scott, 1932.) Speiser mentions a specimen from *P. melanopogon*, also from New Britain, and specimens from New Ireland from *P. celaeno*.

(4) **Cyclopodia albertisi** Rondani, 1878

Syn. *C. pteropus* Rainbow, 1904

Length 4–5 mm. Specimens from the Kei Islands 5–6 mm. Colour reddish to dark brown.

Head similar in general as in *C. sykesii* (Fig. 12). The anterior part of the vertex is covered with 25–30 short setae, not counting those at the anterior dorsal margin. About 40 such setae on the vertex in the specimens from the Kei Islands. Thorax and legs as in *C. sykesii*.

Abdomen, male as in *C. sykesii* in general. Only 12–20 minute spines on tergite 1 of which the posterior ones are not much longer than the anterior spines. 5–6 rather short setae at the posterior lateral corners of tergite 2. Sternites 1 + 2 with a ctenidium of about 30 teeth, 38 in specimens from the Kei Islands.

Genitalia (Figs. 13*b*, 77*b*). Phallobase with a broad, short hook and about 3 transverse rows of setae on the base. Parameres broadly elliptical, with 2 short hairs. Aedeagus rod 0·75–0·8 mm. long, 0·9 mm. in the specimens from the Kei Islands. The field of spines on the connecting tube dark and dense, as long as the aedeagus rod, or slightly longer.

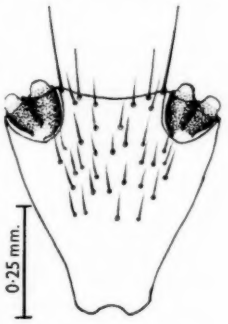


Fig. 12. *Cyclopodia albertisi*: head, dorsal.

Abdomen, female (Fig. 14). Tergite 1 + 2 as in *C. sykesii*, except for the group of minute spines on tergite 1 which are only 12–20 in number. 3–5 moderately long setae at the posterior lateral corners. Dorsum of abdomen covered with minute spines. A group of large spines on a bare area in the middle of the dorsum. There are 4 such spines in most specimens from Australia, only 1–2 or 5–7 in some specimens. There is no group of long setae immediately behind this group of spines as in *C. similis*. 2 groups of long setae at the posterior lateral corners of the dorsum, between spiracles 5 and 6 These groups consist of 6–7 setae in some specimens and only 2 or 3 in others. In a few specimens from the Palau Islands there are 2–4 long setae in the centre of the triangle formed by the anterior group of spines and the two posterior groups of setae. Sternite 1 + 2 with a ctenidium of 36 teeth. Ventral surface covered with short spines which are longer anteriorly at the sides and posteriorly in the middle. Narrow bare spaces in distended specimens between sternites 4 and 5 and sternites 5 and 6. A row of about 10 long setae in the posterior row of sternite 6, opposite the genital plate. This is square, consisting of two roughly triangular lateral sclerites which are covered with short hairs. The sclerites are separated by a membranous area which is wider anteriorly and has 2 branches posteriorly. The posterior margins of each half are rounded and bear about 8 short spines. Some long and some shorter spines in the ventral median corners of the anal sclerites. The specimens from the Palau Islands show a difference from the Australian specimens in the number of large spines on the dorsum of the abdomen of the female, which is statistically significant. 21 out of 43

specimens had only 2 spines, while 12 out of 26 of the Australian specimens had 4 spines.

	No. of spines on the dorsum								
	0	1	2	3	4	5	6	7	
Palau	2	3	21	10	3	2	1	1	Mean: 2·6 ± 0·21 (S.E.)
Australia	—	1	1	5	12	3	2	2	Mean: 4·1 ± 0·26 (S.E.)

No material from the type locality was available, so that the creation of subspecies has to be postponed.

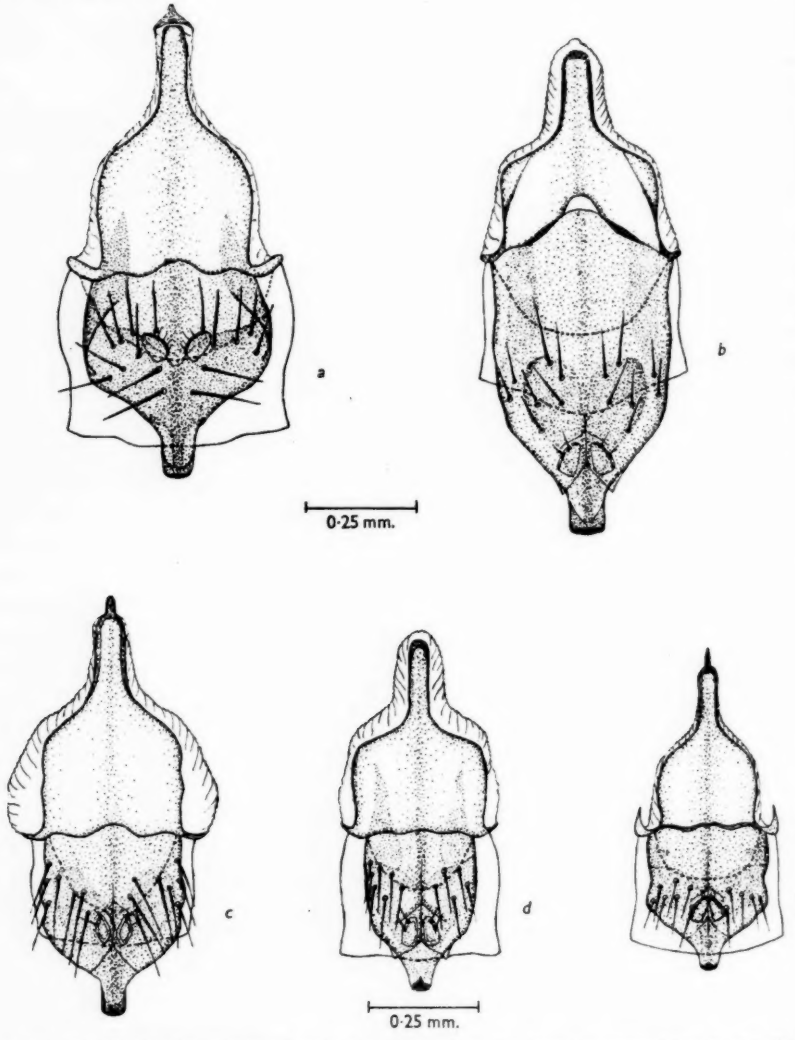
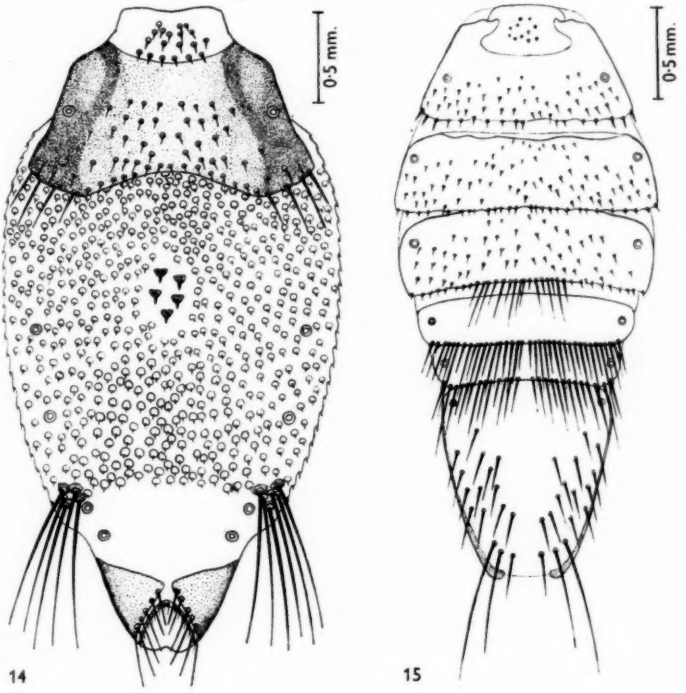
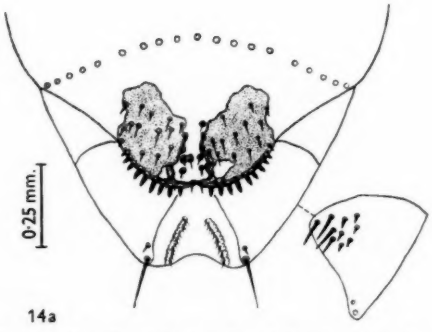


Fig. 13. Basal and phallobase of male genitalia of: *a*, *Cyclopodia oxycephala*; *b*, *C. albertisi*; *c*, *C. bougainvillensis* sp.nov.; *d*, *C. ponapensis* sp.nov.; *e*, *C. inclita*.

Distribution and hosts. MOLUCCAS: Goram (type locality). NEW GUINEA: Kei and Salawati Islands. AUSTRALIA: N.E. Queensland, Sydney, *Pteropus gouldi*, *P. conspicillatus*, *P. poliocephalus*. PALAU ISLANDS: *Pteropus pelewensis* (Strong). (See map no. 2.)





Figs. 14, 15. Fig. 14. *Cyclopodia albertisi*: female, abdomen, dorsal. *a*, genital plate.
Fig. 15. *C. australis* sp.nov.: male, abdomen, dorsal.

(5) **Cyclopodia australis** sp.nov.

This species occurs together with *C. albertisi* and resembles it in many respects, particularly in the female, so that it has not been separated from *C. albertisi* in the past.

Description. Resembling *C. albertisi* in general, differing as follows:

Head. Only 8–10 short setae on the vertex between the eyes, not counting those at the anterior dorsal margin.

Thorax. Notopleural setae absent in 4 specimens, in one specimen a small notopleural seta on one side only. Cover of haltere groove as in *C. albertisi.*

Abdomen, male (Fig. 15). The marginal rows of tergites 2 and 3 consist of very short spines, like those on the surface of the tergites. 2–4 spines at the lateral posterior corners of tergite 2 longer than the others. There are no long setae in the marginal rows of these tergites at all. Tergite 4 with a group of moderately long setae in the middle third of the margin and short spines in the lateral thirds. Tergites 5 and 6 with continuous marginal rows of moderately long setae. There may be a small gap in the middle of tergite 6. Surface of tergite 2 covered with short spines in its posterior half, tergites 3 and 4 completely covered with short spines, tergites 5 and 6 bare. Tergites 5 and 6 of about equal length, while in *C. albertisi* tergite 6 is shorter than 5. Sternite 5 with a row of 12–15 club-shaped spines in the middle of the hind margin.

Genitalia. The connecting tube is wholly covered with short, triangular spines (Fig. 77*h*). The aedeagus rod is rather thick and tapers apically to a fine point. It is 0·9 mm. long including the apical point which is bent backwards in some specimens.

Abdomen, female. There is no anterior group of large spines in the centre of the dorsum in the single female examined. The groups of setae at the posterior lateral corners of the dorsum consist of 9–11 long setae which are more widely spaced than in *C. albertisi.* Genital plate square, with an indistinct, narrow, median membranous stripe covered with isolated short setae. Ventral surface of anal sclerites completely covered with short spines up to the anus.

Material examined. Townsville, Queensland, *Pteropus gouldi*, 1 ♂ (Lee) type. Darwin, Northern Territory, 2 ♂ 1 ♀ (para and allotype). Mary River near Darwin 1 ♂. (British Museum, Nat. Hist.).

(6) **Cyclopodia oxycephala** Bigot, 1860

The species has been re-described by Falcoz (1923).

Length 4–5 mm. Colour dark brown.

Head similar as in *C. albertisi.* The group of short hairs on the vertex consists of 25–30 hairs which extend only a short distance behind the eyes, leaving the greater posterior part of the vertex bare.

Thorax and *legs* as in *C. sykesii.* 3–5 notopleural setae. The rings on the tibiae lie close together in the basal half, the apical ring at half the length of the tibia.

Abdomen, male, as in *C. albertisi.* Sternites 1 + 2 with a ctenidium of 36–42 teeth. None of the specimens examined had 48 teeth as stated by Falcoz.

Genitalia (Fig. 13*a*). Phallobase with a short, broad hook, deeply concave basally and with a W-shaped row of setae on the base. Aedeagus rod 0·8–0·9 mm. long. The field of spines of the connecting tube covers about three-quarters of the length of the rod in the resting position. Parameres elliptical with 2 or 3 short hairs.

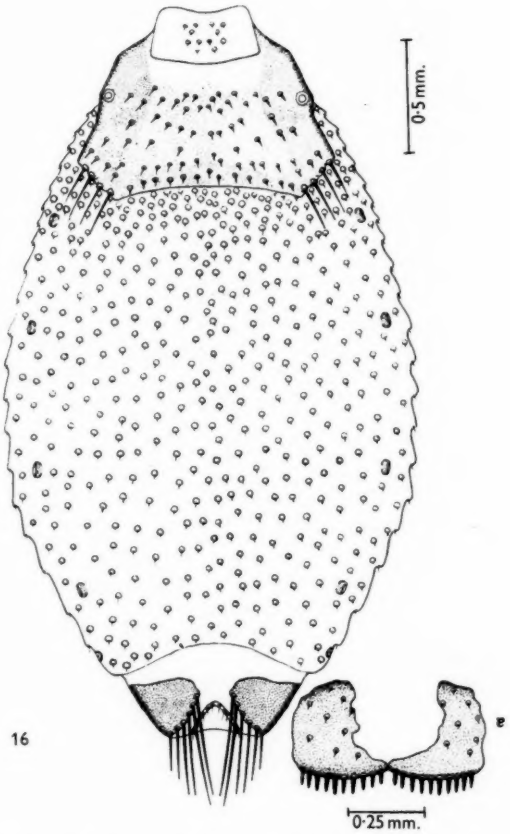


Fig. 16. *Cyclopodia oxycephala*: female, abdomen, dorsal. *a*, genital plate.

Abdomen, female (Fig. 16). Tergite 1 + 2 as in *C. albertisi*. Dorsum of abdomen covered with minute spines without any longer spines or setae. Ventral surface covered with spines which are longer than on the dorsum. Some slightly longer spines in the middle of the marginal row of sternite 5 and about 8 long setae in the middle of the marginal row of sternite 6. Narrow bare spaces discernible between the sternites in distended specimens. Genital plate broader than long, consisting of 2 lateral sclerites with 6–8 short hairs on the surface and a square median membranous area. A row of about 10 short spines at the posterior margin in each half. About 5 small spines in the median ventral corners of the anal sclerites.

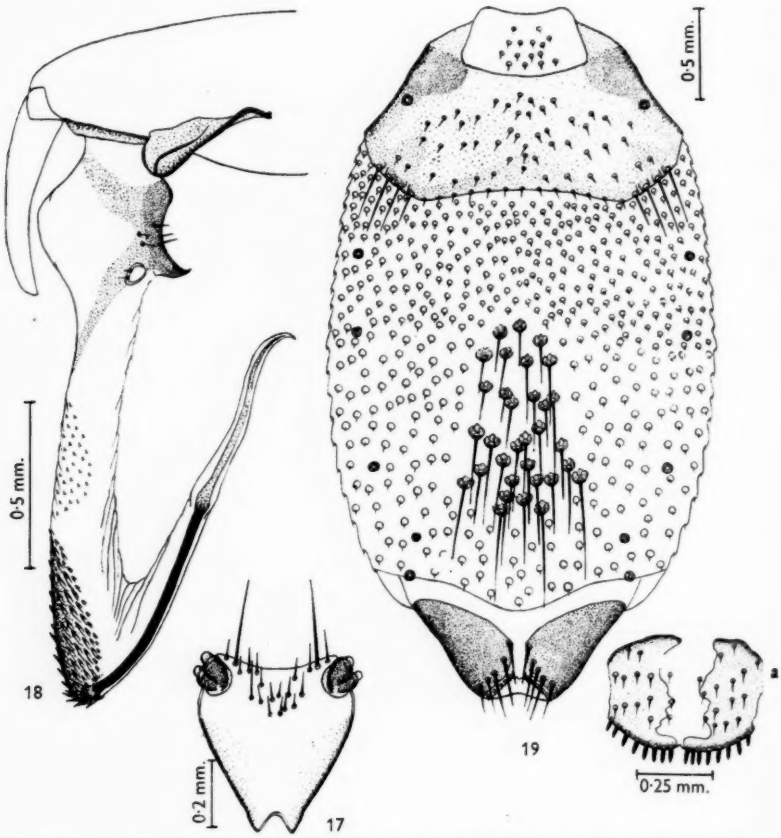
Distribution and hosts. New Caledonia, Loyalty Islands (type locality). New Hebrides.

Material examined. Loyalty Islands (type series). NEW CALEDONIA: Ubatche, Paita, *Pteropus ornatus* (Nicholson); Moindy, *P. geddiei*. NEW HEBRIDES: Malekula, Ounua (Cheesman); Havanna Harbour, Efate Island (Dumbleton); Hog Harbour, Espiritu Santo. *P. eotinus, P. geddiei* (Baker); same locality and hosts, about 50 specimens (Fritts, Johnson, Wharton).

(7) **Cyclopodia inclita** Falcoz, 1927

Length 3·5–4·5 mm. Colour reddish to dark brown.

Head (Fig. 17). Vertex with a triangular group of about 12 short hairs between the eyes, bare in the posterior two-thirds. A longer and 1 or 2 shorter setae on each side of the anterior dorsal margin, only short setae in some specimens. Labella of labium as long as the theca.



Figs. 17–19. *Cyclopodia inclita*. Fig. 17. Head, dorsal. Fig. 18. Male, genitalia extended. Fig. 19. Female, abdomen, dorsal; *a*, genital plate.

Thorax and *legs* as in *C. oxycephala*. 2 to 3 notopleural setae.

Abdomen, male, as in *C. albertisi*, with short setae at the lateral posterior corners of tergite 2. The marginal setae on tergites 3–6 are shorter than in *C. albertisi* and *C. oxycephala* in most specimens, but not in all. Abdominal ctenidium with 36 teeth. Sternite 5 with the posterior margin only slightly convex.

Genitalia (Figs. 13*e*, 18). The genitalia resemble those of *C. oxycephala*, but the basal bulb of the connecting membrane is absent. Instead of a basal bulb, which lies apical to the phallobase in other species in the extended position, there is here a ventral widening of the connecting tube near its junction with the abdomen. The aedeagus rod is very short, 0·6–0·7 mm. long, with a long, membranous apical prolongation. The field of spines is also very short, covering only about half the length of the rod (Fig. 77*g*).

Abdomen, female (Fig. 19). 4–5 short setae at the lateral posterior corners of tergite 2; 10–16 minute spines on tergite 1. Dorsum covered with minute spines on sclerotized pigmented bases. In the middle of the dorsum an oblong group of 20–30 long, thin setae in transverse rows of 4–6. In some specimens a few spines in the anterior rows of the group. Several rows of minute spines behind the group of long setae. Only 4–5 short setae around the anal frame on the dorsal side. Ventral surface covered with short spines. Some longer spines in the marginal row of sternite 5 and 8–10 long setae in the middle of the marginal row of sternite 6, opposite the genital plate. Genital plate rectangular, broader than long, consisting of 2 lateral sclerites which are covered with small hairs. They are separated by a median membranous stripe. About 8 short spines at the posterior rounded margin in each half. A few minute spines at the median ventral corners of the anal sclerites.

Distribution and hosts. Samoa: Upolu, Apia. *Pteropus tonganus, P. samoensis* (type series) (Buxton and Hopkins). Fiji Islands: Viti Levu (Lever); Tamavua, Vuna Taveuni (Symes).

(8) **Cyclopodia minor** Speiser, 1901

Speiser's description is incomplete in essential characters. Scott (1932) re-examined specimens of the type series and gave figures of the abdomen of male and female. Two male specimens from Ponam in the Admiralty Islands are ascribed to this species. The description of the male is based on these specimens and that of the female is given after Speiser and Scott.

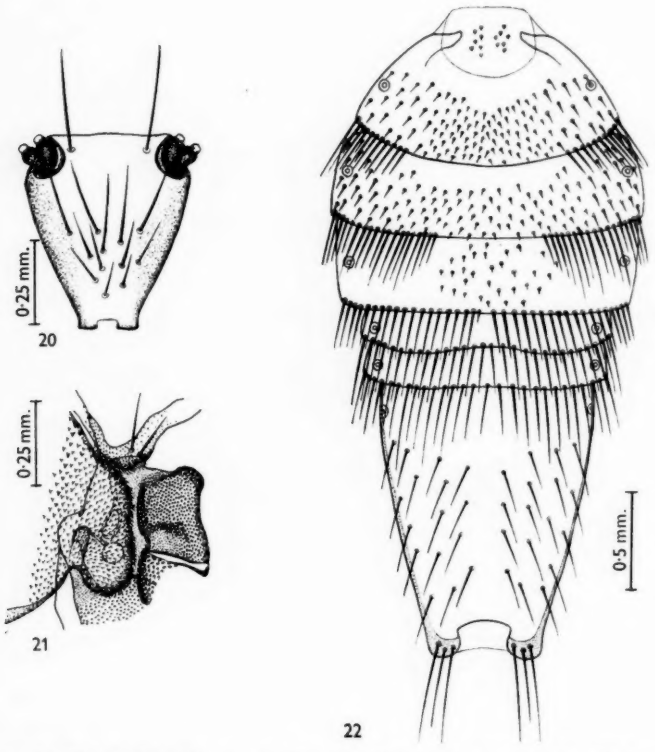
Length 3·7–4 mm. Colour dark brown.

Head (Fig. 20). 2 long setae at the anterior dorsal margin near the eyes. A group of 10–12 rather long setae in the posterior half of the vertex. Anterior part bare. Eyes large as in the *sykesii* group.

Thorax in general as in the *sykesii* group, but the haltere groove resembles the arrangement in the *pembertoni* group (Fig. 21). The cover of the groove is attached to the notopleural suture along its whole length, not only in its posterior part, and does not cover the groove completely. The lateral bulge is reduced, but formed as in the *pembertoni* group. There are 2 notopleural setae.

Abdomen, male (Fig. 22). Tergite 1 with a group of 12–15 minute spines. Tergite 2

with 8–10 moderately long setae at the lateral parts of the posterior margin. Surface covered with short spines except in the anterior lateral corners. Tergite 3 with a similar marginal row with a wide gap in the middle. (Speiser states that the marginal rows of all tergites are continuous.) Surface covered with small spines except in some patches anteriorly. Tergites 4–6 with continuous, dense marginal rows with a small gap in the middle of tergite 4. A group of very small



Figs. 20–22. *Cyclopodia minor*. Fig. 20. Head, dorsal. Fig. 21. Haltere groove. Fig. 22. Male, abdomen, dorsal.

spines in the middle of the surface of tergite 4, tergites 5 and 6 bare. Tergites 5 and 6 are much shorter than the preceding tergites. Anal segment broad, truncate, with moderately long setae in the lateral parts of the dorsal surface. Abdominal ctenidium with 33–38 teeth. Sternites 3–4 with dense marginal rows of moderately long setae and short spines on the surface. Sternite 5 much longer than 4, with convex posterior margin which bears 6 barrel-shaped spines in the middle and moderately long setae laterally. There is a premarginal row of 7 to 8 long setae on each side and short spines on the posterior part of the surface.

Genitalia. Claspers broad, 4 times as long as thick at the base. They bear two long and some shorter setae dorsally near the base. Phallobase with short apical

hook and with a double row of setae on the base. Parameres elliptical with 3–4 short hairs. Aedeagus 0·75 mm. long. Field of spines nearly as long as the rod.

Female. Tergite 1 + 2 broad, its surface covered with short hairs. Posterior margin shallowly sinuate. About 7 rather long setae at the lateral posterior corners. Dorsum of abdomen covered with minute spines. Some longer setae on the pleurae anteriorly and a transverse row of longer setae, which is interrupted in the middle, at the hindmargin of sternite 4. Ventral surface otherwise covered with short spines. No long setae on the dorsum. Anal segment conical, with a row of long setae around the anus. Genital plate suboblong, with curved hindmargin which bears about 6 short spines in each half. The genital plate is sclerotized at both sides, membranous in the middle. There are 2 irregular rows of very short spines obliquely across each half of the plate.

This species is intermediate in several respects between the *sykesii* and *pembertoni* group. The eyes are large as in the *sykesii* group. The arrangement of the setae on the vertex differs from that in both groups. The haltere groove has a flap which covers it nearly completely, but is attached along its whole length. The marginal rows of the tergites of the male abdomen are broadly interrupted on tergites 2 and 3 as in the *sykesii* group and are continuous on tergites 4–6 as in the *pembertoni* group. Scott identified specimens from the Solomon Islands tentatively as *C. minor* and this was published by Thompson (1937). Re-examination of these specimens has shown, however, that they belong to a new species of the *tenuis* group and they are described below as *C. solomonarum* sp.nov.

Distribution and hosts. New Britain, Matupi Island. *Pteropus admiralitatum.* (Dahl) (type series.)

Material examined: Admiralty Islands: Ponam Island. *P. neohibernicus*, 2 ♂♂ (Wharton).

(9) **Cyclopodia bougainvillensis** sp.nov.

Length 4–4·5 mm. Colour dark brown.

Head. 2 long and several shorter setae on the anterior dorsal margin. A group of 20–25 short hairs on the vertex between the eyes which leaves the posterior two-thirds of the vertex bare as in *C. oxycephala.* Eyes large. Labella of labium as long as the theca.

Thorax and *legs* as in *C. oxycephala.* 2–3 notopleural setae.

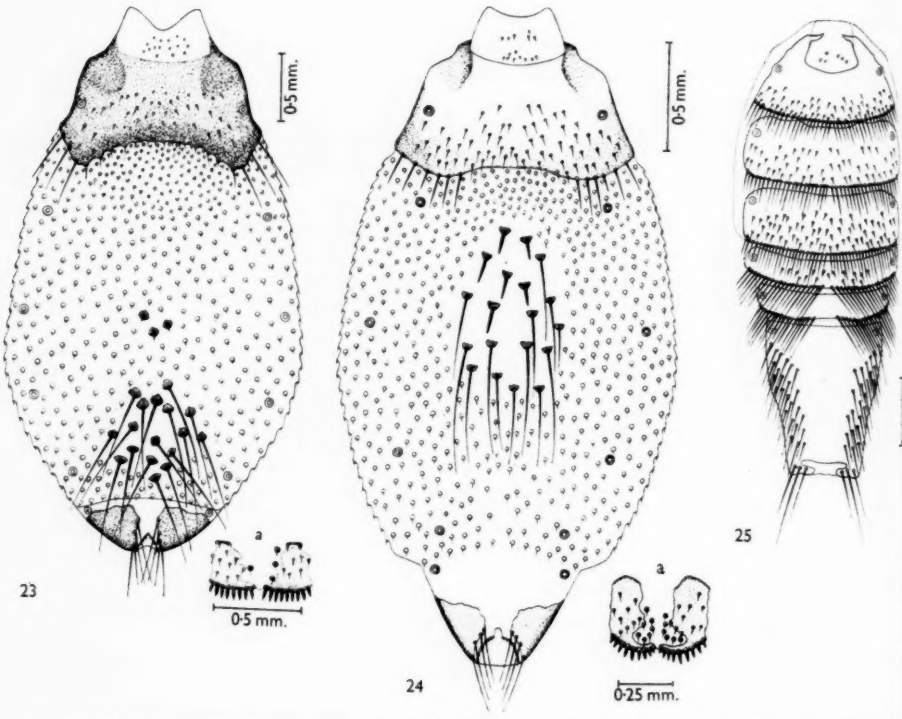
Abdomen, male. Tergite 2 with 4–5 short setae in the posterior lateral corners. Otherwise as in *C. oxycephala.*

Genitalia. Claspers 4–5 times as long as broad at the base. Phallobase with broad apical hook, a deep basal incision and a W-shaped row of about 16 setae on the base (Fig. 13*c*, 77*e*). Aedeagus rod 0·8 mm. long. Field of spines on the connecting tube covering three-quarters of the length of the rod. Parameres elliptical with 3–4 short hairs.

Abdomen, female (Fig. 23) resembling *C. inclita* in having only a single group of long setae on the dorsum. The group consists, however, of only 11–15 setae in most specimens as against 25–30 in *C. inclita.* The group is situated farther back than in *C. inclita* near the posterior border of the spiny area of the dorsum.

Anteriorly to the group of long setae there is in most specimens a group of 2–3 spines which are larger than the others covering the dorsum, but much smaller than, for example, in *C. albertisi*. One specimen had only 1 such spine, 1 had 4, and they were absent in one specimen. Genital plate rectangular, consisting of 2 lateral sclerites with a wide median membranous stripe. 8–10 short spines at the posterior margin in each half.

This species differs from *C. inclita* in the male genitalia which resemble those of *C. oxycephala*, and in the female in the presence of a group of spines anteriorly to the group of long setae. The number of long setae is much smaller than in *C. inclita* and their position is different.



Figs. 23–25. Fig. 23. *Cyclopodia bougainvillensis* sp.nov.: female, abdomen, dorsal. *a*, genital plate. Fig. 24. *C. ponapensis* sp.nov.: female, abdomen, dorsal. *a*, genital plate. Fig. 25. *C. pembertoni*: male, abdomen, dorsal.

Material examined. SOLOMON ISLANDS: Bougainville, Cape Torokino, *Pteropus grandis*, 2 ♂, 2 ♀, type series (U.S. National Museum); *P. mahaganus*, *Pteralopex anceps*, *Pipistrellus ponceleti*, 4 ♂, 4 ♀, paratypes (Carver, Fritts, Johnson, Wharton); Guadalcanal, *Pteropus rubianus*, 2 ♂, 3 ♀ (Nicholson); Balesuna, Guadalcanal, *Pteropus* sp., 1 ♀ (Lever).

(10) **Cyclopodia ponapensis** sp.nov.

Length 4·5 mm. Colour dark brown.

Head. 2 long and several short setae on the anterior dorsal margin. A group of 18–25 short hairs on the anterior part of the vertex leaving the posterior half bare. Eyes large. Labella of labium longer than the theca by one-third.

Thorax as in *C. albertisi*. 1–2 notopleural setae.

Abdomen, male. About 16 minute spines on tergite 1, the posterior spines longer than the anterior ones. About 8 rather short spines on the posterior lateral corners of tergite 2. About 35 teeth in the abdominal ctenidium. Posterior margin of sternite 5 convex with about 8 short club-shaped spines in the middle.

Genitalia (Fig. 13*d*, 77*f*). Claspers broad. Phallobase with broad apical hook. Two groups of 5–6 setae on the base. Aedeagus rod 0·7 mm. long, field of spines nearly as long as the rod, very similar to that of *C. albertisi*, but shorter.

Abdomen, female (Fig. 24). Tergite 2 with 6–8 short setae in the posterior lateral corners. Dorsum covered with small spines which are larger than in other species. In the middle of the dorsum an oblong group consisting of 5–6 very long and strong spines anteriorly and 8–10 long setae posteriorly. On the ventral side the posterior margin of sternite 5 is well marked by longer setae and a bare space behind them. About 6 long setae in the middle of the marginal row of sternite 6. Genital plate rectangular, with 2 lateral sclerites separated by a membranous stripe which widens anteriorly and posteriorly. About 10 short spines on the posterior margin in each half.

This species resembles *C. inclita* in the female, but differs from it in the chaetotaxy of the vertex and the group of setae on the dorsum of the abdomen. The male of *C. ponapensis* resembles *C. albertisi* in the chaetotaxy of the vertex and in the genitalia.

Material examined. Caroline Islands: Ponape Island, Colonia, *Pteropus molossinus*. 3 ♂, 3 ♀ (type series) (Dybas, Thomas, Hurlbut). (Chicago National History Museum.)

PEMBERTONI GROUP

(11) **Cyclopodia pembertoni** Scott, 1932

Length 3·5–4 mm. Colour reddish brown.

Head. Vertex and anterior margin bare. Eyes small, with ocelli less deeply separated than in the *sykesii* group, with shallow, small lenses. Antennae rounded anteriorly. Palps widening apically, with long terminal seta, 3–4 short setae at the dorsal margin, and a denser row at the ventral margin. Labella of the labium nearly twice as long as the theca. 5–6 minute spines at the posterior margin of the theca in each half.

Thorax. Median ventral suture indistinct in the middle. Angle of oblique sutures about 90°. Mesonotum slightly wider posteriorly with a small, rounded posterior plate. The posterior part of the notopleural sutures forms a flap-like expansion which covers the haltere groove partly from the median side. Opposite this flap there is a large rounded bulge which narrows the opening of the haltere groove from

the side so that only a narrow gap remains open (Fig. 3*b*). Two rather long notopleural setae. Thoracic ctenidium with about 15 blunt teeth. Legs rather short, tibiae 4 times as long as wide in the middle, tapering towards both ends. Tibial rings close together, in the basal half of the tibia.

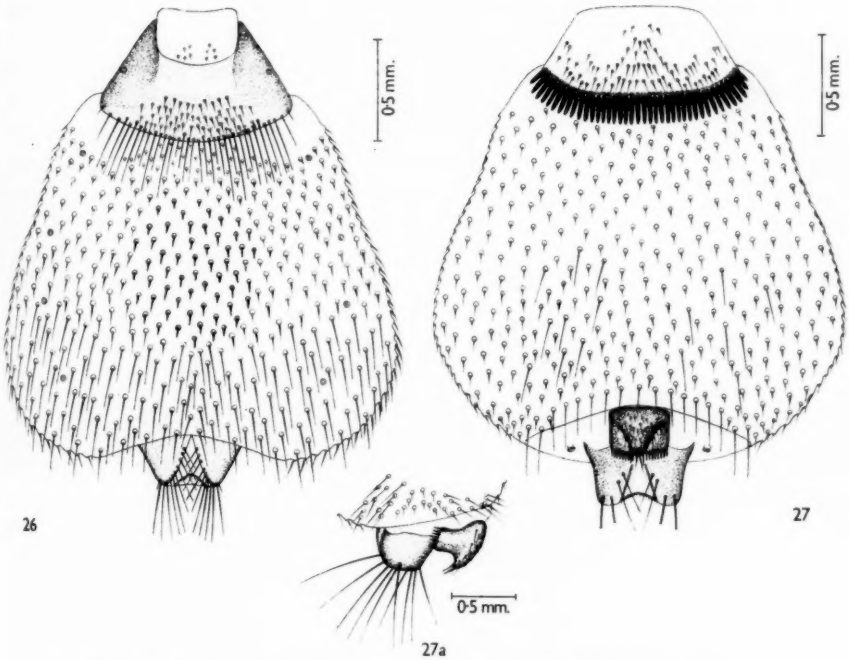
Abdomen, male (Fig. 25). Tergite 1 with 6–8 minute spines. Tergites 2 to 6 with continuous marginal rows of moderately long and shorter setae. A small gap in the middle of the rows of tergites 5 and 6. Tergite 2 with about 3 rows of short spines on the surface near the posterior margin. Tergites 3 and 4 covered with short spines in the posterior two-thirds and tergite 5 with an irregular double row of short spines in the middle. The marginal setae of tergites 5 and 6 are considerably longer than those of tergites 2 to 4. Tergite 6 bare. Anal segment conical, flattened, covered with moderately long setae except for a median stripe, which widens anteriorly. Sternite 1 + 2 very short, with a ctenidium of about 40 teeth. Surface covered with short spines except for two patches in the middle. Sternites 3 and 4 with dense marginal rows of moderately long setae which are shorter in the middle. Surface covered with short spines. There is a premarginal row of upright setae which are as long as, or longer than, the setae of the marginal rows in the lateral thirds. The posterior margin of sternite 4 is concave. Sternite 5 not much longer than 4, with slightly convex posterior margin which bears about 10 short, club-shaped spines in the middle and a series of thin setae between them. Lateral parts of the posterior margin with moderately long setae. Surface covered with short spines and short setae in the posterior half and one or two premarginal rows of long upright setae laterally.

Genitalia. Claspers nearly parallel-sided up to the middle, tapering from there to a sharp pigmented point. Basal arc with sloping shoulders and broad anterior process. Phallobase triangular, with a short hook and a V-shaped row of about 10 setae on the base. The anterior ventral processes of the phallobase are very long, ribbon-shaped and unite at about half length of the connecting tube, forming a long process. Parameres elliptical with 2–3 minute hairs and a small basal process. Aedeagus rod 0·7 mm. long, thick, with an apical membranous part. Connecting tube with a field of spines which is as long as the rod.

Abdomen, female (Figs. 26, 27). Abdomen much wider than long, widest posteriorly, trapezoid in dorsal view in distended specimens, flattened dorsoventrally. Tergite 1 + 2 triangular with a convex posterior margin which bears a row of long setae. These are shorter and more widely spaced laterally. The posterior median part of the surface is covered with short setae and there are several long premarginal setae laterally.

Dorsum covered with raised, cone-shaped, pigmented tubercles which bear short spines anteriorly and long setae posteriorly. These setae are longest in the median posterior part of the dorsum. The transition from short spines to long setae is abrupt in the middle of the dorsum where a row of long setae follows a row of short spines. It is gradual at the sides where moderately long setae expand onto the pleurae. The anal segment is truncate, sharply set off from the abdomen and bears a row of long setae around the anus. Sternite 1 + 2 as in the male, very short. Ventral surface covered with similar tubercles as the dorsum. These bear

short setae and there are a number of longer setae, some of which probably indicate the limits of segments. There are, however, no recognizable bare intersegmental spaces. 4 to 6 of these longer setae stand in rows which are probably the hindmargins of sternites 4 and 5, and 4–6 such setae stand at the sides of the marginal row of sternite 6. The genital plate is sclerotized throughout with a basal curved horn which is covered with short spines. The apical part of the plate is bare and bears a row of 9–13 short spines in each half of the nearly straight hindmargin. There is a group of about 10 short spines on the medium ventral corners of the anal sclerites which is generally covered by the genital plate.



Figs. 26, 27. *Cyclopodia pembertoni*. Fig. 26. Female, abdomen, dorsal.
Fig. 27. Female, abdomen, ventral. *a*, genital plate and anal segment, lateral.

Distribution and hosts. Fiji Islands: Lami, Viti Levu (Pemberton), type series; Kalabu caves, Insectivorous bat (Symes); Colombo cave, Suva, Fiji, *Notopteris macdonaldii* (Wonder). (Crane Pacific Expedition 1929.)

The apparently regular occurrence of a *Cyclopodia* on an insectivorous bat is exceptional for the genus.

(12) **Cyclopodia macracantha** sp.nov.

Length 4 mm. Colour dark brown.

Head (Fig. 28*a*). Eyes small as in *C. pembertoni*, but with prominent, rather small lenses. Ocelli deeply separated. Anterior dorsal margin with a row of about

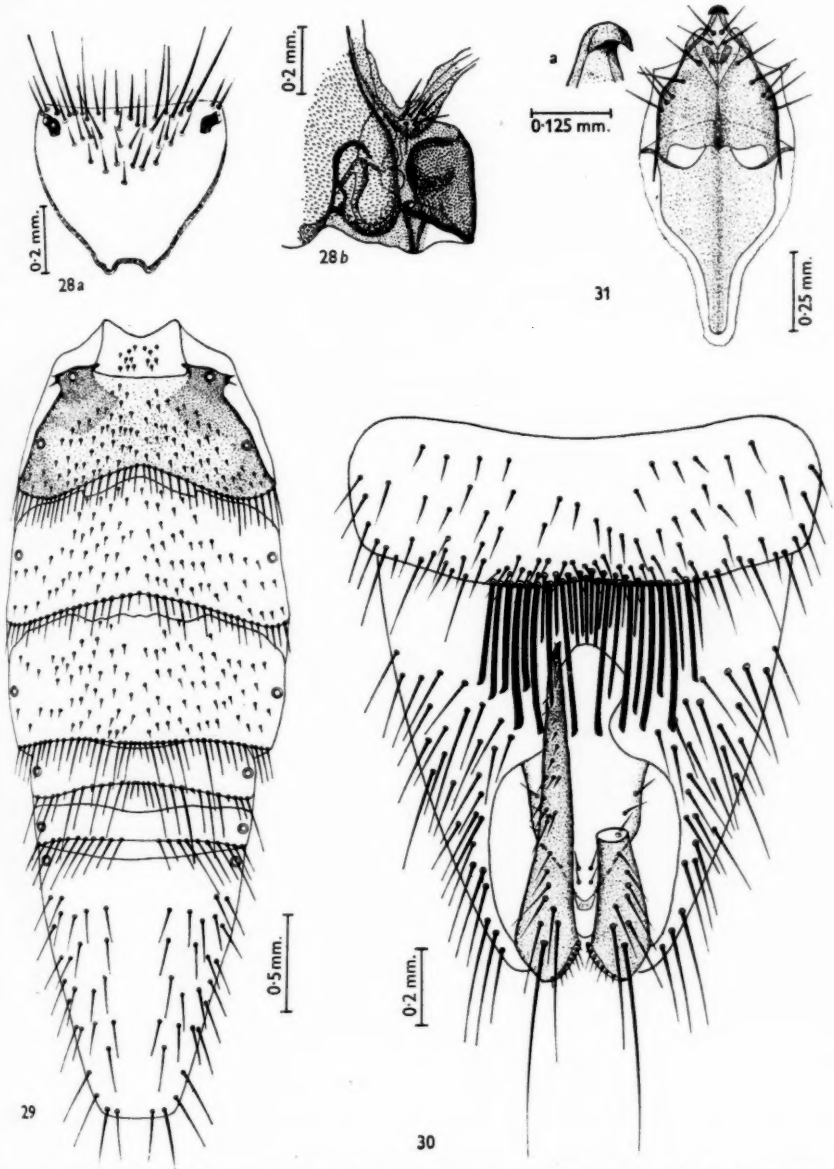
10 setae which are shorter in the middle. Behind the marginal row, between the eyes, about 20 short hairs in 2 to 3 rows. Vertex otherwise bare. The anterior marginal row continues onto the anterior ventral margin and there are 6–10 setae on the genae. Palps parallel sided, tapering towards the tip. There are 3 short recumbent hairs near the base on the dorsal edge and 4 upright, more widely spaced setae further distally. Terminal seta as long as the palp. The row on the ventral edge of the palp consists of 8–10 closely standing setae which grow longer towards the tip. Labella of the labium slightly longer than the theca.

Thorax slightly wider than long. Angle of oblique sutures about 90°. Dorsal surface similar as in *C. pembertoni*, except that there are about 8 notopleural setae in a dense group. Haltere groove similar as in *C. pembertoni*, but the median flap is broader, covering the groove nearly completely (Fig. 28*b*).

Abdomen, male (Figs. 29, 30). Spiracle 1 is included in tergite 1 + 2 and does not lie in the pleural membrane as in most other species. There are 1–3 short spines lateral to it. About 15 minute spines in the membrane of tergite 1. Marginal rows of tergites 2–6 continuous. The marginal row of tergite 2 consists of very short spines in the middle and of longer spines laterally in some specimens and of longer spines throughout in others. A triangular field of short spines covers the posterior part of the surface. The marginal rows of tergites 3 and 4 are similar and the setae vary also very much in length in different specimens. Surface covered with short spines which stand more closely in the middle. Tergites 5 and 6 very short, bare on the surface, with marginal rows which consist of long upright setae laterally and some shorter setae between them. A small gap in the middle of the rows. Anal segment broad, conical, covered with moderately long setae dorsally, leaving a median stripe bare. Sternite 1 + 2 rectangular with a ctenidium of about 40 blunt teeth. Surface densely covered with short spines which are short anteriorly and much longer laterally and posteriorly. Sternites 3 and 4 with slightly concave hind-margin which bears a dense row of moderately long and shorter setae. Surface covered with short spines anteriorly and short setae posteriorly and laterally. There is a premarginal row of upright setae. Sternite 5 shorter than 4, rectangular, with straight posterior margin. There is a row of about 18 very long, peculiar spines in the middle of the posterior margin. Some of these spines are longer than the segment, others are shorter. Longer and shorter spines alternate. The spines do not taper, but have a truncate tip with a short point which is directed laterally in each half. Anterior to this group, very close to it, there is another row of about 8 spines which are about half as long as those of the marginal row, standing close together. These spines have normal points. Lateral to the marginal group of long spines there are some ordinary short setae. Some shorter setae and short spines cover the posterior lateral part of the surface. Anal segment ventrally also covered with short setae. The frame formed by the anal segment around the genital area has two lateral processes anteriorly which are more prominent than in other species, forming a narrow neck for the basal part of the frame in which the tips of the claspers rest.

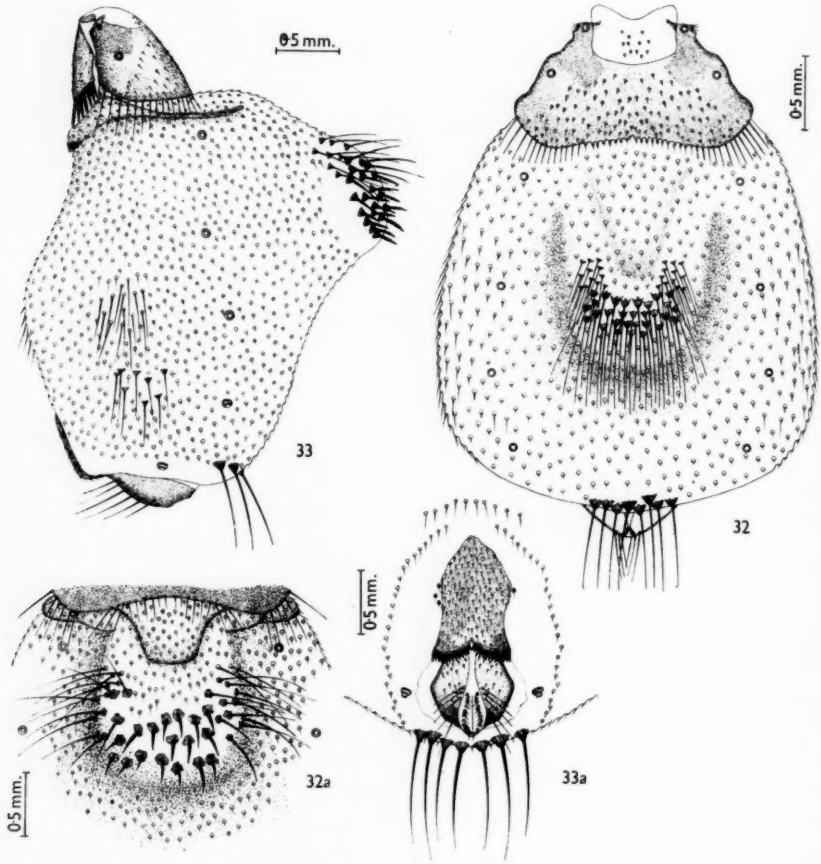
The lateral parts of the tergites and sternites are coloured more darkly than the middle in most specimens.

Genitalia (Fig. 31). Claspers very slender, 6–7 times as long as wide at the base, tapering to a long pigmented tip. A long seta and 2 shorter ones dorsally near the base and a double row of short setae and short spines in the basal half which become



Figs. 28–31. *Cyclopodia macracantha* sp.nov. Fig. 28. *a*, head, dorsal; *b*, haltere groove. Fig. 29. Male, abdomen, dorsal. Fig. 30. Male, abdomen, sternite 5 and genital area. Fig. 31. Male, genitalia, basal arc and phallobase. *a*, hook of phallobase.

gradually shorter towards the tip, where they are minute. Basal arc triangular with a long anterior process. Phallobase with a long hook which has a spatulate or bifid tip. A V-shaped row of setae on the base, reaching nearly to the base of the hook. 5–7 setae in each arm of the V. Aedeagus rod simple, 1·1 mm. long, pointed apically with a short apical membranous part. The field of spines of the connecting tube is as long as the rod.



Figs. 32–33. *Cyclopodia macracantha* sp.nov. Fig. 32. Female, abdomen dorsal. *a*, dorsal hump, dorsal in a specimen with large spines instead of setae. Fig. 33. Female, abdomen, lateral (specimen with spines on dorsal hump instead of setae). *a*, genital plate and anal segment.

Abdomen, female (Figs. 32, 33). Tergite 1 + 2 with a marginal row which consists of short or very short spines in the middle. They become gradually longer towards the sides and there are moderately long setae in the lateral corners. Surface covered with short spines in the posterior half, reaching further anteriorly in the middle. Dorsum covered with short spines. In the middle of the dorsum, on a

hump in distended specimens, a crescent-shaped group of about 50 long setae. These setae are very long and thick in the middle of the group, standing on widely spaced, darkly pigmented tubercles, and smaller and standing more closely laterally. In some specimens some or all of the large setae are replaced by very long, thick spines (Fig. 32*a*). At the posterior margin of the spinose area of the dorsum, in front of the anal segment, a row of about 8 long setae on large tubercles similar to those in the middle of the dorsal group. Sternite 1 + 2 as in the male with a ctenidium of 40–44 teeth. Venter covered with short setae, and there are rows of 6–8 longer, upright setae in the marginal rows of sternites 3–5. Sternite 6 with deeply concave posterior margin and only short setae in the marginal row. Genital plate (Fig. 33*a*) shield-shaped with the point anteriorly, and covered with short spines except in a stripe along the posterior margin. Posterior margin curved in each half and bearing there about 15 closely standing spines. Ventral corners of the anal sclerites covered with short spines.

Distribution and hosts. Solomon Islands: Fatura, Isabel Island, *Dobsonia inermis*, 1 ♂ (Lever), type (British Museum, Natural History); Tunnibuli, Isabel Island, *Pteropus* sp., 2 ♂, 2 ♀, Crane Pacific Expedition, 1929, allotype and paratypes (Chicago Natural History Museum); Webster cave, Kolombangara, Crane Pacific Expedition, 1929, 1 ♂, 1 ♀; Cape Torokino, Bougainville, *P. mahaganus*, *P. solomonis*, 2 ♂, 2 ♀ (Carver, Johnson), paratypes (U.S. National Museum); Lunga, Guadalcanal, *P. woodfordi*, 1 ♂ (Lever), paratype (British Museum, Nat. Hist.).

GREEFFI GROUP

(13) **Cyclopodia greeffi** Karsch, 1884

Syn. *Cyclopodia rubiginosa* Bigot, 1891

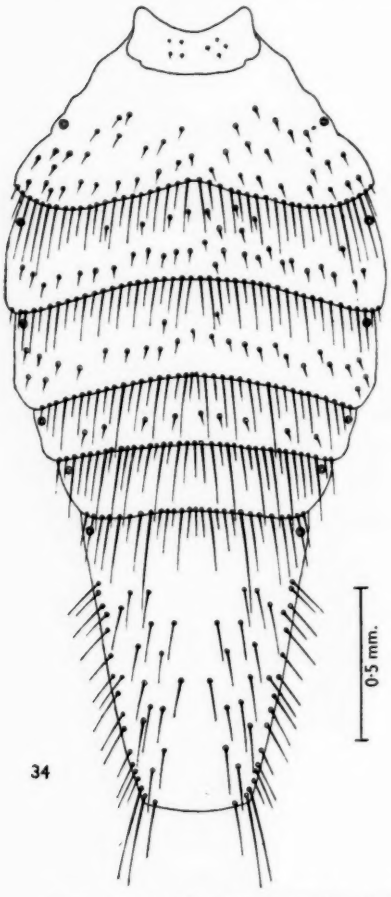
Length 4 mm. Colour reddish brown.

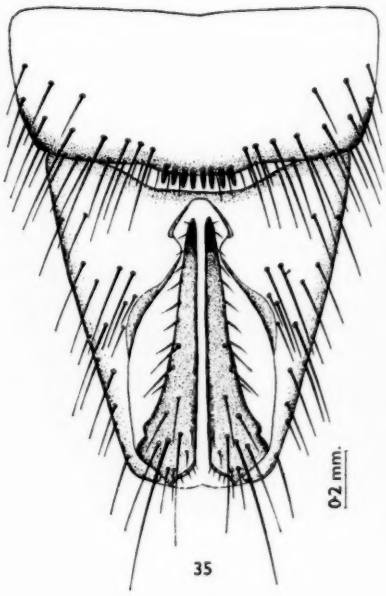
Head. Vertex bare. Two setae at the anterior dorsal margin and a row of short setae beginning near the eyes on the anterior ventral margin. Genae bare. Eyes large with well-separated ocelli and prominent lenses. Palps with a row of about 5 short setae at the dorsal margin and about 8 at the ventral margin. Terminal seta as long as the palp. Labella of the labium slightly longer than the theca. A row of 5 minute spines at the posterior margin of the theca in each half.

Thorax (Fig. 2*a*) slightly wider than long. Median sternal suture indistinct in the middle, widening in the anterior part towards the middle and then tapering to a point. Oblique sutures forming an angle of 90°. The spines of the sternal plate are smaller than in *C. sykesii* and there is a bare stripe along the median suture in some specimens. The posterior margin of the sternal plate is bare except for a few short setae at the sides. Mesonotum wide. Only one notopleural seta. Haltere groove open, cover rudimentary, with a small anterior rounded process (Fig. 3*c*). Tibiae cylindrical, slightly compressed laterally, 5–5·5 times as long as wide, with 3 oblique rings in the middle (Fig. 2*b*).

Abdomen, male (Figs. 34, 35). About 10 minute spines on tergite 1. The marginal rows of all tergites are continuous and consist of longer and shorter setae. The setae on tergites 5 and 6 are longer than on the anterior tergites. The surface is

covered with short spines in the posterior half of tergite 1+2 and on tergite 3. There is an irregular double row on tergite 4 and tergites 5 and 6 are bare. In some specimens a few setae in the middle of tergite 5. Tergites 5 and 6 are shorter than the anterior tergites, but not as short as in *C. sykesii*. Anal segment long, conical, with moderately long setae on the dorsal surface except on a median stripe which





Figs. 34, 35. *Cyclopodia greeffi*. Fig. 34. Male, abdomen dorsal. Fig. 35. Male, abdomen, sternite 5 and genital area.

widens anteriorly. Sternite 1+2 rectangular, with a ctenidium of 40–44 thick, blunt teeth. One or two rows of very short spines along the anterior margin in the middle and longer, upright setae at the sides. 2 or 3 rows of longer spines along the posterior margin, mainly in the middle. Sternites 3 and 4 with marginal rows of short setae. Surface of sternite 3 covered with short spines anteriorly in the middle, and short setae laterally and posteriorly. There is a premarginal row of upright setae at both sides of the surface. Sternite 4 similar, but the short spines in the

middle of the surface are absent. Sternite 5 longer than 4, with a convex hind-margin which bears a row of 6–10 spines in the middle (only 4 in one specimen). These spines are not club-shaped, and are longer and thinner than in *C. sykesii*. Short setae lateral to the group of spines. Surface with a premarginal row of upright setae at both sides and one or two similar rows at the sides.

Genitalia (Figs. 4, 35). Claspers long and slender, tapering to a long, pigmented point, 6·5 times as long as thick at the base. A long and 2 shorter setae dorsally

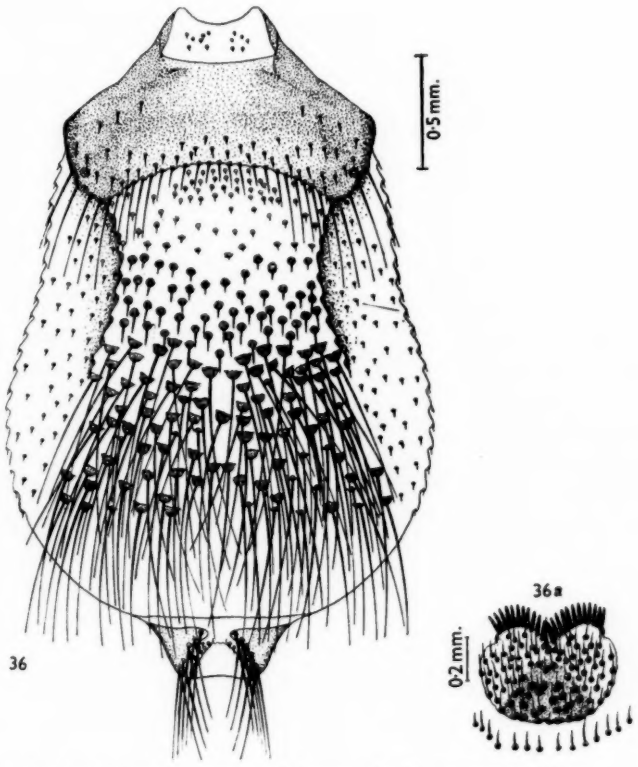
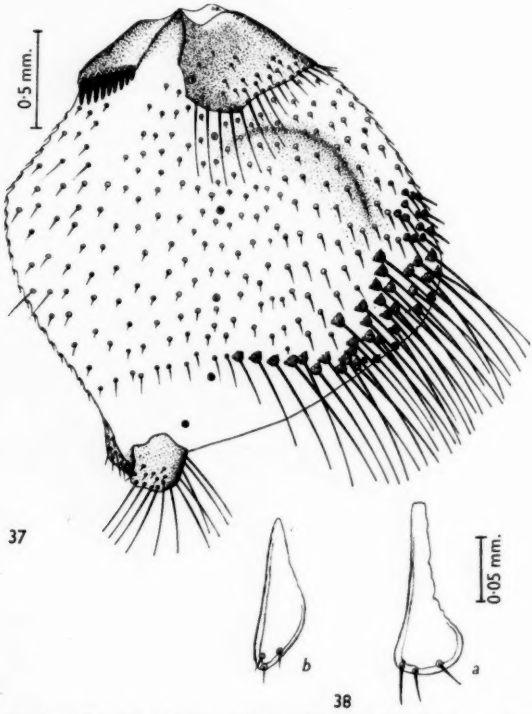


Fig. 36. *Cyclopodia greeffi*: female, abdomen, dorsal. *a*, genital plate.

near the base and a double row of short setae and short spines on the dorsal surface. Phallobase with a long, slender hook and 2 groups of 5 to 6 thin setae on the base. Basal arc with a long conical anterior process and a posterior concavity. Parameres narrowly triangular with rounded posterior end which bears a few minute hairs. Aedeagus rod long (1·2 mm.), tapering to a fine point. The rod articulates basally with a short U-shaped sclerite which forms a frame around the distal end of the connecting tube. The field of spines covers only about half of the length of the rod and the spines are shorter than in *C. sykesii* and not pigmented.

Abdomen, female (Figs. 36, 37). Tergite 1 + 2 large, triangular with slightly con-

cave posterior margin which bears a row of setae which are much longer laterally. Short setae among the longer ones in the middle. The surface of tergite 1+2 is covered with short spines in the middle of the posterior part and longer spines posteriorly and laterally. Dorsum covered with short spines which are very small anteriorly in the middle and become longer posteriorly. The posterior part of the dorsum is covered with a rectangular group of very long setae, about 70–80 in 7–8 rows. The posterior row reaches further laterally than the anterior ones. The



Figs. 37, 38. Fig. 37. *Cyclopodia greeffi*: female, abdomen, lateral.
Fig. 38. Parameres of male. *a*, *C. greeffi*; *b*, *C. greeffi arabica*.

last 3 rows in front of the group of setae consist of very long and thick spines. The pleurae are covered with short spines anteriorly and short setae posteriorly. Between the group of long setae and the anal segment the abdomen is bare. The spiracles are small, about half the diameter of those of *C. sykesii*. The anal segment is short, truncate, with 2 rows of long setae around the anus.

The shape of the abdomen in distended specimens is truncate posteriorly, not ovoid as in most species of the *sykesii* group. It appears nearly square in dorsal view. The ventral surface is more or less convex, but the dorsal surface is bent, the anterior and posterior halves standing at right angles to each other, so that the abdomen appears triangular in profile. The long setae stand on the posterior half of the dorsum (Fig. 37).

Sternite 1 + 2 as in the male, but longer. Ventral surface covered with short spines. 3 or 4 long setae in the marginal row of sternite 3 on each side. The rows of spines on sternite 6 are shorter than on the preceding sternites and do not reach the sides. The genital plate is formed by a transversely elliptical field of spines on sclerotized bases which is incompletely divided in the middle. The posterior margin is more strongly sclerotized and bears a curved row of about 15 long, blunt spines in each half. There are about 100 spines on the plate in 6–8 rows. The bases of the spines are fused in the median anterior part of the field. A few short spines on the median ventral corners of the anal sclerites which are covered by the genital plate.

Distribution and hosts. Details of the material examined (about 250 specimens), localities and hosts have been given in a previous paper (1957). *C. greeffi* has been found in a belt across Central Africa, between 10° lat. south and north. In West Africa it occurs farther to the north, nearly up to 20° lat. north. Its main host is *Eidolon helvum* which occurs throughout Africa from south of the Sahara to South Africa. Only a few specimens were found on *Rousettus angolensis* and *Epomophorus* sp.

(13*a*) **Cyclopodia greeffi arabica** Theodor, 1954

This subspecies resembles *C. greeffi* in most respects. In the female, the group of long setae on the dorsum of the abdomen consists of only 35–50 setae in 5–6 rows. The group of spines on the genital plate consists of about 65 spines in 5–6 rows. In the male the only difference noted is in the shape of the parameres which have a pointed distal end (Fig. 38).

Distribution and hosts. South East Arabia: Yemen, Ta'izz; Aden, Lahej, *Eidolon sabaeum* (Yerbury, Hoogstraal).

(14) **Cyclopodia dubia** (Westwood) 1835

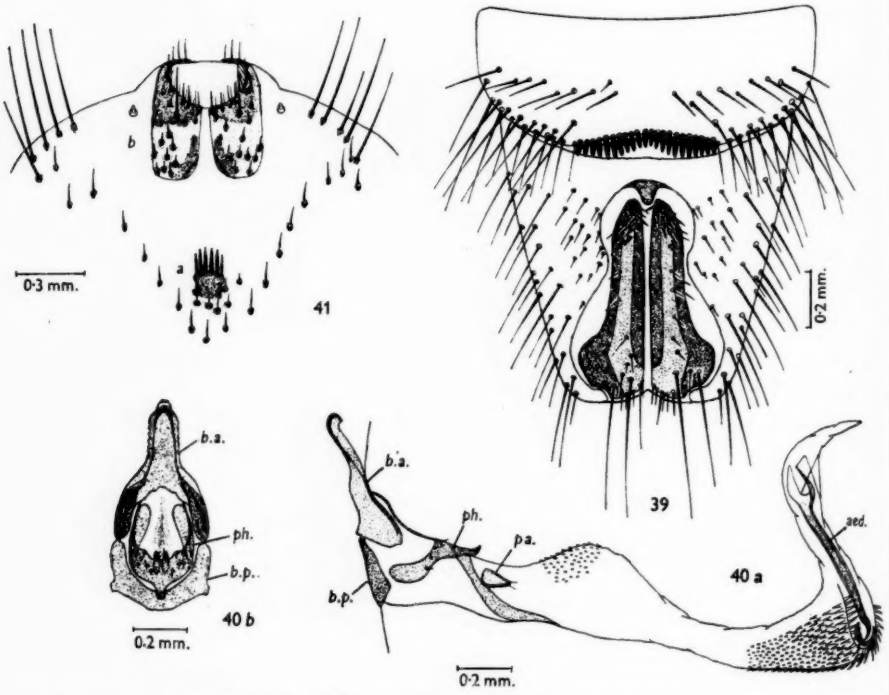
Length 3·5–4 mm. Colour brown.

Head. Vertex and anterior margin bare. A row of short setae on the anterior ventral margin with a longer seta posteriorly. Eyes markedly smaller than in *C. greeffi* and ocelli less deeply separated. Labella of labium 1·5 times as long as the theca.

Thorax slightly wider than long, rounded anteriorly. Thoracic ctenidia with about 16 thick, blunt spines. One notopleural seta. Median ventral suture indistinct in the middle. Oblique sutures forming an angle of 95°. Haltere groove open. Legs rather short, with 3 oblique rings in the middle of the tibiae.

Abdomen, male (Fig. 39). Tergite 1 with about 10 minute spines. All tergites with continuous marginal rows of longer and shorter upright setae. The setae are longer on the posterior tergites. Surface of tergite 1 + 2 with 2–3 rows of short spines in the posterior part. Tergites 3–6 bare. Tergites 5 and 6 much shorter than 4. Anal segment long, nearly cylindrical, covered dorsally with upright setae except in a median stripe. Sternite 1 + 2 trapezoid, short, with a ctenidium of about 40 blunt teeth, 3–4 rows of short spines in the posterior half of the surface and 1–2 rows of very small spines along the anterior margin. Sternites 3 and 4

with marginal rows of longer and shorter setae. There are 4 rows of short spines in the middle and longer setae at the sides of the surface of sternite 3. Sternite 4 similar, but there are only 1–2 rows in the middle and the setae of the premarginal row are longer. Sternite 5 not much longer than 4. A row of 15–20 long, thick, but not club-shaped spines in the middle of the hindmargin and long setae laterally. There is a premarginal row of very long, upright setae with a wide gap in the middle



Figs. 39–41. *Cyclopodia dubia*. Fig. 39. Male, sternite 5 and genital area. Fig. 40. Male, genitalia. *a*, extended; *b*, basal arc and phallo-base; *pa.*, paramere. Other letters as in Fig. 4. Fig. 41. Female, genital plate and anal segment.

opposite the spines and a row of short setae in the middle of the surface, also with a gap in the middle.

Genitalia (Fig. 40). Claspers thick and short, straight, with rounded, pigmented tips, which bear a group of strong spines. There are 3 long setae dorsally near the base and a few short setae on the dorsal surface. There is a rounded process at the anterior ventral margin of the anal segment opposite the tips of the claspers. Basal arc Y-shaped, with a long, blunt anterior process and a deep posterior concavity. Basal plate strip-like, curved. Phallobase short, with a deep concavity anteriorly and a short, thick hook. Two groups of 5–6 setae at the base. Parameres elliptical with a few short hairs. The basal bulb of the connecting tube is covered with scale-like teeth dorsally and posteriorly. The field of spines is about as long as

the aedeagus rod. Aedeagus rod short, thick (0·5 mm.) with a semicircular basal sclerite. The rod is bifid distally and forms a partial frame around the gonopore.

Abdomen, female (Figs. 41, 42). The abdomen appears rectangular in dorsal view in distended specimens and is flattened dorsoventrally to some extent. The dorsal angle characteristic for *C. greeffi* is absent. Tergite 1 + 2 has a marginal row which

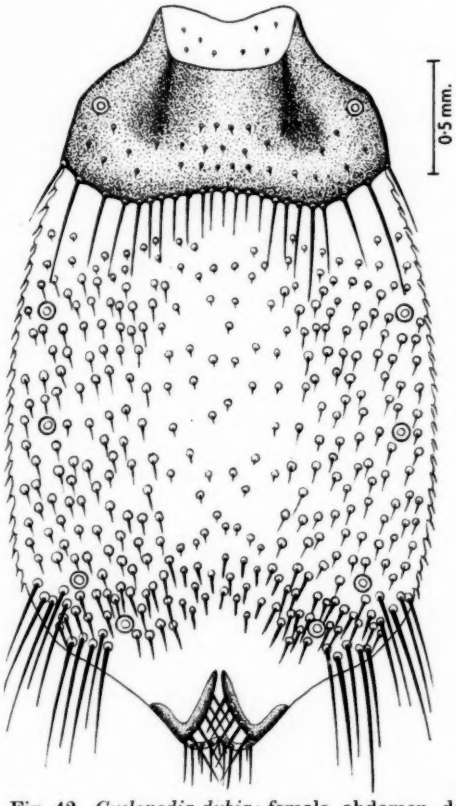


Fig. 42. *Cyclopodia dubia*: female, abdomen, dorsal.

consists of longer setae laterally and shorter setae in the middle. 3–4 rows of short spines in the posterior part of the surface. Dorsum of the abdomen with minute spines in a broad median stripe and with larger spines at the sides of the dorsum and at the pleurae. There are 2–3 rows of still larger spines at the posterior margin of the spinose area. There are 2–3 rows of setae at the posterior part of the pleurae, between spiracle 5 and 6 and between 6 and 7. Anal segment very short, consisting of a narrow strip dorsally on each side. These strips widen ventrally into rectangular plates which are covered with short spines. Anteriorly the plates dissolve into separate bases for the spines. A row of setae surrounds the anal frame. Spiracles small as in *C. greeffi*. Sternite 1 + 2 as in the male but longer. Ventral

surface uniformly covered with short spines, so that the limits of the segments are scarcely discernible. There are about 12 rows of short spines on sternite 3 and 2 long upright setae in the middle of the posterior row of the surface. Sternites 4 and 5 much shorter, with only 2 or 3 rows of short spines and 4 long vertical setae in the marginal row or in the posterior row of the surface. Sternite 6 concave posteriorly with only 2 or 3 rows of short spines. Genital plate small, triangular, with 6–10 long spines posteriorly. These spines are as long as, or longer than, the plate. A few spines on sclerotized bases stand closely to the plate anteriorly.

There are 2 triangular, darkly pigmented spots at the anterior margin of the pleurae, just behind the posterior margin of tergite 1 + 2 and sternite 1 + 2, where the inner surface of femur 3 touches the abdomen. In cleared preparations under high magnification these spots show a reticulate structure. Nothing like it has been observed in other species of Nycteribiidae.

Distribution and hosts. Madagascar, mainly on *Eidolon dupreanum*. Exact data of the material examined have been given in a previous paper (Theodor, 1957).

TENUIS GROUP

(15) **Cyclopodia tenuis** Schuurmans Stekhoven & Hardenberg, 1938

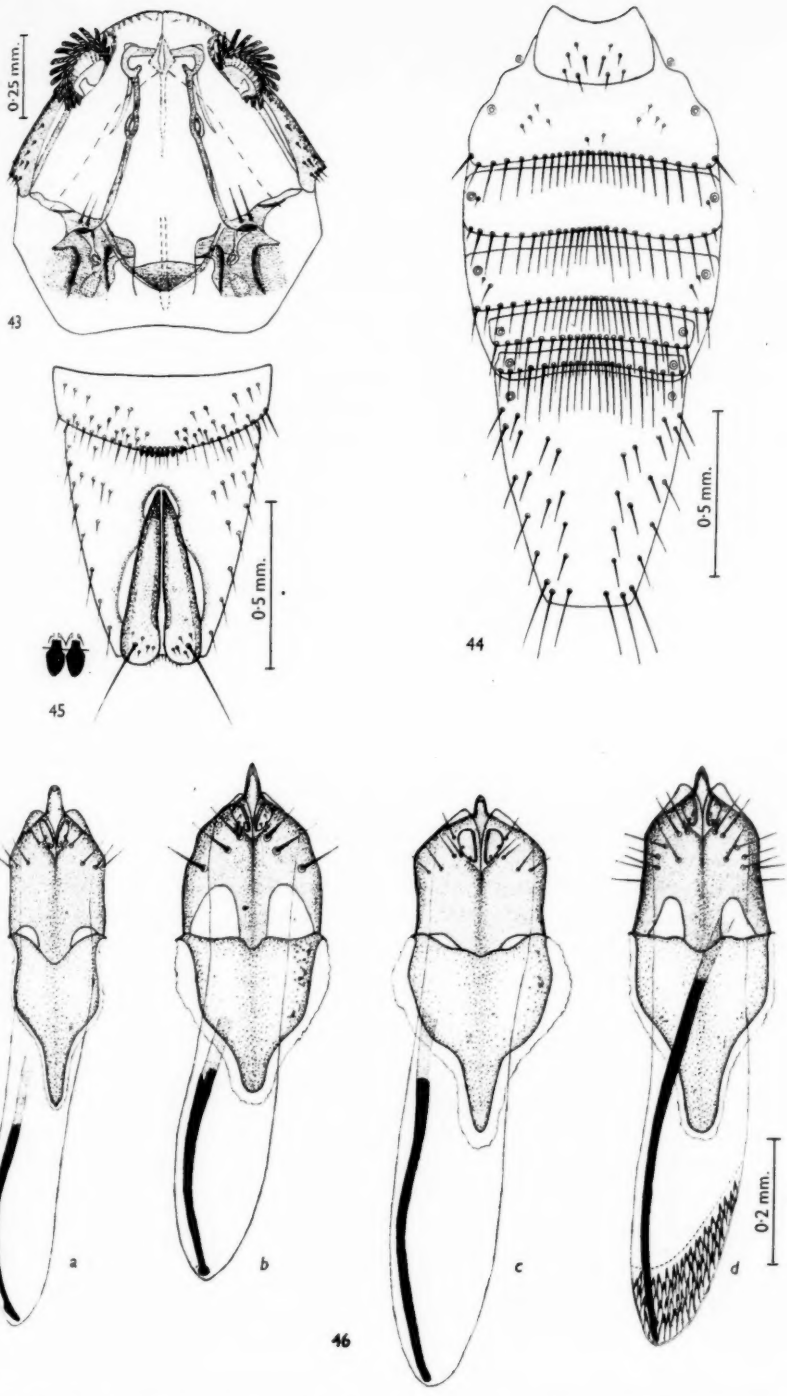
Only the male was described by Schuurmans Stekhoven. The female is here described for the first time.

Length 2–2·5 mm. Colour yellowish.

Head. Vertex bare, 2 short setae at the anterior dorsal margin. Eyes with deeply separated ocelli with small lenses. A row of 6–8 short setae along the anterior ventral margin. Genae bare. Palps with broadly rounded apical end and short terminal setae. A single seta at the dorsal edge near the tip and one or two short setae near the base. About 6 short setae at the ventral edge. Labella of labium as long as the theca. 4 minute spines at the posterior margin of the theca in each half.

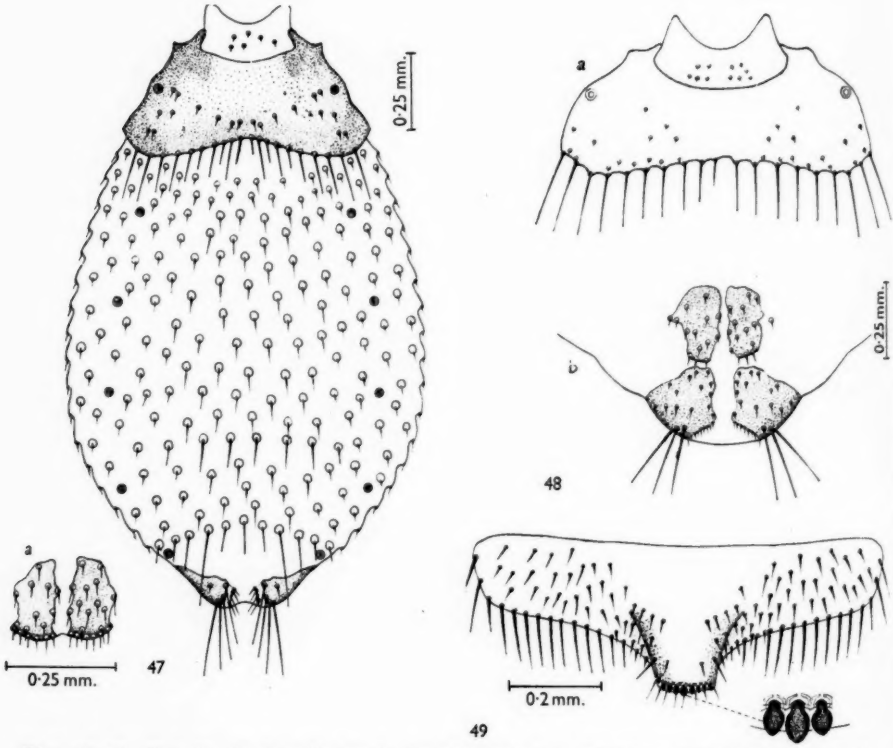
Thorax (Fig. 43) as long as wide, rounded and much narrower anteriorly between the ctenidia which have about 15 blunt teeth. Median ventral suture indistinct in the middle. Oblique sutures forming an angle of about 80°. Sternal plate covered with rather long spines and minute hairs among them. Only such minute hairs anterior to the oblique sutures between the ctenidia. Posterior margin of the sternal plate bare, only 3 short setae stand close to the margin at the sides. 1 or 2 notopleural setae. Haltere groove open. The sclerite connecting the thoracic ctenidium with coxa 2 is narrowly triangular and has a single row of short setae anteriorly and 2–3 rows posteriorly. Anterior coxae very long, three-fifths of the length of the front femur. Legs rather short. Tibiae 1 slightly shorter than femur 1. Tibial rings in the middle of the tibia. Tibia 4 times as long as wide, slightly compressed laterally.

Abdomen, male (Figs. 44, 45). Tergite 1 + 2 triangular. A group of 8–10 spines on tergite 1 which are much longer than in other species of the group. A continuous marginal row of widely spaced short setae at the posterior margin. Only a few



Figs. 43–46. *Cyclopodia tenuis*. Fig. 43. Thorax, dorsal. Fig. 44. Male, abdomen, dorsal. Fig. 45. Male, abdomen, sternite 5 and genital area. Fig. 46. Male genitalia of: *a*, *Cyclopodia tenuis*; *b*, *C. solomonarum* sp.nov.; *c*, *C.*(?) *inflatipes*; *d*, *C. truncata* sp.nov.

short spines at the sides of the surface, near spiracle 2. Tergites 3 and 4 with similar marginal rows, bare on the surface except for 1 or 2 short spines at the sides near the spiracles. Tergites 5 and 6 very short, with similar marginal rows and bare on the surface. Anal segment long and broad, conical, covered with short setae except in a median stripe. Sternite 1 + 2 trapezoid, with a ctenidium of 45 teeth. Surface covered with longer spines posteriorly and much shorter spines anteriorly. Sternites 3 and 4 with marginal rows of short setae, surface of sternite 3 wholly



Figs. 47–49. Fig. 47. *Cyclopodia tenuis*: female, abdomen, dorsal. *a*, genital plate. Fig. 48. *C.*(?) *inflatipes*: female, abdomen. *a*, tergite 2, and *b*, anal segment and genital plate. Fig. 49. *C. truncata* sp.nov.: male, abdomen, sternite 5.

covered with short spines which are longer posteriorly. Such spines only on the posterior two-thirds of the surface of sternite 4. Sternite 5 not longer than 4, with convex posterior margin which bears 8 short, club-shaped spines in the middle and a row of fine setae behind them. Short setae at the lateral parts of the posterior margin and some short spines in the lateral posterior parts of the surface.

Genitalia (Fig. 46*a*). Claspers straight, tapering to a pigmented tip, about 5 times as long as wide at the base. A long and several short setae dorsally near the base and some minute spines near the tip. Basal arc triangular, with pointed anterior process. Phallobase triangular, with a narrow, rather long apical hook.

A V-shaped row of 8 setae at the base. Parameres narrowly elliptical, with 3 minute hairs. Aedeagus rod very short and thin (0·33 mm.), simple, widening slightly towards the tip. The field of spines of the connecting tube consists of long unpigmented spines and is longer than the aedeagus rod.

Abdomen, female (Fig. 47). Tergite 1 + 2 as in the male, with concave hindmargin. The lateral setae of the marginal row are longer and stand wider apart than the median ones. A few short spines in the lateral part of the surface and a few along the hindmargin in the middle. The dorsum of the abdomen is covered with short spines anteriorly and laterally. A transverse row of longer spines in the posterior part of the dorsum. The posterior row of the spinose area has 4–5 setae in the middle which are longer than the rest. Anal segment very short, rounded, with a row of long setae around the anal frame and a few short spines on the surface of the sclerites. Sternite 1 + 2 as in the male. Venter covered with short spines which stand more closely anteriorly. There are no long setae indicating the limits of segments but narrow bare spaces between some of the sternites in the very distended specimen examined. Genital plate square, consisting of two sclerites which are covered with short spines and a narrow membranous stripe between them. A row of 5 to 7 longer, thin spines at the posterior margin in each half. A few small spines at the ventral median corners of the anal sclerites.

Distribution and hosts. Java. Types from Tulung Agung. Moluccas.

Material examined. Java, *Macroglossus minimus*, 1 ♂; West Java, *Megaderma spasma trifolium*, 1 ♀ (Balston & Shortridge); Yilolo, Halmahera, Moluccas, *Macroglossus minimus*, 1 ♀; *M. minimus* does not occur in the Moluccas according to Laurie and Hill (1954) and the species concerned is probably *M. lagophilus*.

(16) **Cyclopodia** (?) **inflatipes** Speiser, 1901

This species was described from a single female from New Guinea. The description would fit any species of the *tenuis* group. The following description is based on specimens from New Guinea, which are provisionally considered as belonging to this species.

Length 2–2·5 mm. Colour yellowish brown.

Head. Vertex bare, two short setae at the anterior dorsal margin, close to the eyes. Eyes with deeply separated ocelli. 6–8 short setae at the anterior ventral margin. Genae bare. Palpi with a short terminal seta and 4–5 short setae on the ventral and dorsal margin. Labella of labium as long as the theca. 4 minute spines at the posterior margin of the theca in each half.

Thorax. Very similar to that of *C. tenuis*. Slightly wider than long. 3 notopleural setae. Legs as in *C. tenuis*.

Abdomen, male. Very similar to that of *C. tenuis*, except that there are a few scattered short setae in the middle of the surface of tergites 2–4 and the group of minute hairs on tergite 1 consists of 10–15 very short hairs. Sternite 5 more convex posteriorly than in *C. tenuis* and the short setae on the surface are more numerous.

Genitalia (Fig. 46*c*). Basal arc broad, with long anterior process. Phallobase

with a short apical process and a V-shaped row of about 10 setae on the base. Aedeagus rod longer than in *C. tenuis* (0·45 mm.). Field of spines of the connecting membrane longer than the rod, consisting of unpigmented spines. Parameres triangular, with 3 short setae.

Abdomen, female (Fig. 48). Very much as in *C. tenuis* The short setae on the surface of tergite 2 are more numerous and the marginal setae are more widely spaced in the middle of the row. The posterior row of long setae on the dorsum is more widely spaced with a gap in the middle. Genital plate with 2 lateral sclerites which are wider anteriorly and are covered with short thin spines. Only 3 to 4 such spines at the posterior margin in each half.

Material examined. NEW GUINEA: Papua, Mt Dayman, Maneau Range, 3 ♂, 1 ♀ (Van Deusen).

This species is very closely related to *C. tenuis* and this may eventually prove a subspecies of it.

(17) **Cyclopodia truncata** sp.nov.

Length 3 mm. Colour yellowish.

Head as in *C. tenuis*. Vertex bare, 2 setae at the anterior dorsal margin. A row of about 10 short setae at the anterior ventral margin. Palps broadly rounded anteriorly with a row of 7–8 short setae at the ventral and dorsal margin. Terminal seta short. Labella of labium as long as the theca. 7–8 minute spines at the posterior margin of the theca in each half.

Thorax slightly wider than long, as in *C. tenuis*. Angle of oblique sutures 90°. Haltere groove open. One notopleural seta. Legs short, as in *C. tenuis*. Front coxae two-thirds the length of the front femur.

Abdomen, male (Fig. 49). Only 4 minute spines on tergite 1. Marginal rows of tergites 2–4 continuous, uniform, consisting of short setae and some short spines. Posterior lateral part of the surface of tergite 2 covered with short spines, about 30 on each side and a few in the middle near the marginal row. Tergites 3 and 4 with 10–15 short spines on the lateral part of the surface. Tergites 5 and 6 very short, bare on the surface and with marginal rows of setae which are longer than on the anterior tergites. Anal segment as in *C. tenuis*. Sternite 5 longer than 4, with a truncate, broad process arising from the middle of the surface, which bears a row of 8 short club-shaped spines and some thin setae between them. There are moderately long setae at the lateral parts of the posterior margin and some spines in the posterior lateral part of the surface.

Genitalia (Fig. 46*d*). Basal arc triangular with broadly rounded anterior process. Claspers thick, 4 times as long as broad at the base. Phallobase triangular, with a short, pointed apical hook. A double, irregular, V-shaped row of about 15 setae at the base. Aedeagus rod simple, thin, 0·6 mm. long. Field of spines of the connecting tube not pigmented, longer than the rod. Parameres elliptical, with 2 minute hairs.

Female unknown.

Material examined. Mioko Island near Duke of York Island, New Britain, *Melonycteris melanops*. 1 ♂ (type) (British Museum, Nat. Hist.).

(18) **Cyclopodia solomonarum** sp.nov.

Length 2·5 mm. Colour yellowish.

Head. A row of 5–8 short setae near the anterior dorsal margin, 2–3 of them on the margin. Palps as in *C. truncata* with short terminal seta and marginal rows of 6-7 short setae, but longer and tapering towards the tip. A row of about 6 short setae at the anterior ventral margin. Labella of labium as long as the theca. A row of 4–5 minute spines at the posterior margin of the theca in each half.

Thorax slightly longer than wide, similar to that of *C. tenuis*. Angle of oblique sutures about 80°. 3 notopleural setae. Haltere groove open. Legs as in *C. tenuis*. Front coxae cone-shaped, very long, three-quarters the length of the front femur.

Abdomen, male. 10 minute spines on tergite 1. Tergite 2 with a marginal row of 3 to 4 longer setae at the lateral corners and short spines in the middle. The posterior half of the surface is covered with short spines and some longer setae in the anterior lateral part. Tergites 3 and 4 with similar marginal rows. (Most of the setae in the middle missing.) 2–3 rows of short spines in the middle of the surface. Tergites 5 and 6 very short, bare on the surface, with uniform, continuous marginal rows of longer setae than on tergites 3 and 4. Anal segment very broad, conical, covered with short setae in the posterior lateral parts, heavily sclerotized, darker brown than the rest of the abdomen. Sternite 1 + 2 with a ctenidium of 42 teeth. Surface covered with short spines posteriorly and laterally. These spines are longest in the anterior lateral corners. The anterior median part of the surface bears 2 rows of very short spines. Sternites 3 and 4 with dense, uniform marginal rows of moderately long setae and short spines on the surface. Sternite 5 slightly longer than 4, with a convex posterior margin which has 8 short club-shaped spines in the middle and moderately long setae laterally. There is a similar premarginal row and some short spines on the lateral posterior parts of the surface.

Genitalia (Fig. 46*b*). Claspers heavily sclerotized, tapering to a blunt point. 4 times as long as broad at the base. Basal arc triangular, broad, with a broadly rounded anterior process. Phallobase with a long slender hook and a V-shaped row of 6 setae at the base. Parameres triangular, with 3–4 minute hairs. Aedeagus rod 0·33 mm. long, with a basal knob, widening and bifid apically. Field of spines of the connecting tube not pigmented, longer than the rod.

Abdomen, female. The posterior part of the abdomen of the only available specimen is damaged and not all details could be made out. Tergite 1 + 2 triangular with 1-2 longer setae at the lateral posterior corners and short spines in the middle of the posterior margin which are not longer than those of the dorsum. 4–5 rows of short spines on the posterior part of the surface. Some of them on the anterior lateral parts longer. Dorsum of the abdomen covered with short spines which are longer posteriorly in the middle Anal segment very short, with a row of short setae around the anal frame. Genital plate consisting of 2 lateral sclerites which are covered with short spines. 4–6 shorter and longer thin spines at the posterior margin in each half.

Material examined. Yandina, Russell Island, Solomon Islands, *Pteropus* sp., 1 ♂, 1 ♀ (Lever), types (British Museum, Nat. Hist.).

Subgenus **Leptocyclopodia** n.subg.

Type species: *Cyclopodia ferrarii* (Rondani), 1878

Diagnosis. Slender insects with long and narrow anal segment in the male. Palps slender, tapering with a long terminal seta. Eyes with ocelli more or less deeply separated (Fig. 50 B). The sclerite connecting the thoracic ctenidium with coxa 2 bears a single row of spines. Haltere groove open. Postspiracular sclerite with or without short spines. Abdominal tergites of the male with continuous rows of setae. Tergite 5 as long as, or longer than, tergite 4. No rows of specialized spines at the hindmargin of sternite 5 of the male.

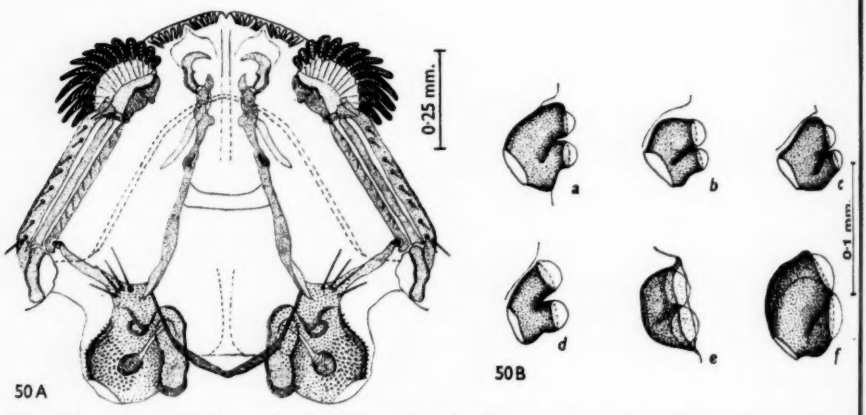


Fig. 50. *Cyclopodia ferrarii.* A, thorax, dorsal. B, eyes of species of the subgenus *Leptocyclopodia*. *a*, *C. ferrarii*; *b*, *C. ferrarii* from Ceylon; *c*, *C. ferrarii palawanensis* subsp.nov.; *d*, *C. brachythrinax*; *e*, *C. simulans*; *f*, *C. macrura*.

Male genitalia: claspers with an armature of spines or specialized setae in some species, without them in others. Aedeagus wholly or partly sclerotized, with an apodeme. Phallobase bifid in some species, with or without an armature of spines dorsally. Segmentation of the female abdomen less reduced than in the typical subgenus. Rows of setae may be present at the posterior margin of some segments on dorsum and venter. Sclerites present on tergite 6 and on sternites 5–7 or 6–7. Sternite 7 not transformed into a genital plate. Anal sclerite present or not.

(19) **Cyclopodia ferrarii** (Rondani) 1878

The species has been re-described by Scott (1914) and Schuurmans Stekhoven & Hardenberg (1938).

Length 2·5 mm. Colour yellowish brown.

Head. 2 setae at the anterior dorsal margin. Vertex bare. 4–5 short spines and 1 longer seta at the anterior ventral margin. A few short spines on the genae. Eyes large, with incompletely separated ocelli and shallow lenses. Palps long and slender, flattened laterally, with long terminal seta and 3–4 setae at the

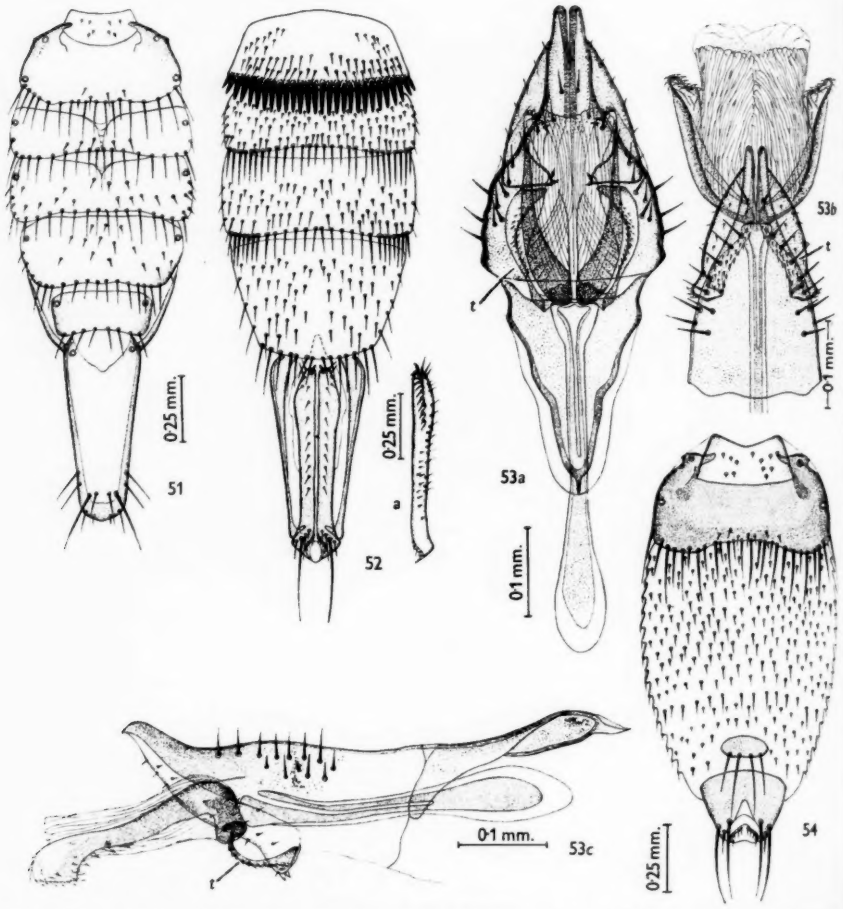
dorsal margin which become longer towards the tip. 2–3 setae at the ventral margin and 1 seta in the middle of the outer surface. Labella of labium as long as the theca. 4 minute spines at the posterior margin of the theca in each half.

Thorax (Fig. 50A). Slightly longer than wide. It resembles the thorax of the *tenuis* group. Angle of oblique sutures about 80°. Thoracic ctenidium with about 13 teeth. The sclerite connecting the ctenidium with coxa 2 consists of 2 narrow strips, the ventral strip bearing a single row of short spines. The posterior margin of the sternal plate is bare except for a few short spines near the margin at the sides. The sternal plate is covered with short spines except anteriorly between the ctenidia where the spines are minute. Haltere groove open. 3 or 4 short notopleural setae in a row. Front coxae very long and slender, about two-thirds of the length of the femur. Front tibia as long as the femur, 5·5 times as long as wide. 3 rings close together in the middle of the tibia. Tibia 2 broader, 4·5 times as long as wide. Length of legs 2·7–2·8 mm. Metatarsus 1 very long (0·85 mm.); metatarsus 3 shorter (0·65 mm.).

Abdomen, male (Figs. 51, 52). Postspiracular sclerite without spines. Tergite 1 + 2 triangular with convex posterior margin. Spiracle 1 lying inside the sclerite. 2 groups of 6–7 minute spines on tergite 1. The marginal row of tergite 2 consists of widely spaced, thick setae of moderate length which have a spiral structure. A premarginal row of about 4 short setae in the middle. Tergite 3 incompletely divided in some specimens. Tergites 3 and 4 with similar continuous marginal rows as tergite 2. 1–3 rows of short setae on the surface. Tergite 5 longer than 4, with a marginal row of shorter, vertically standing setae and 2–3 rows of short setae. Tergite 6 shorter and narrower than 4, with a similar marginal row as 5 and bare on the surface. Anal segment very long and slender, slightly tapering towards the end, 2·5–3 times as long as wide at the base in alcohol specimens. Anterior margin of the dorsal surface deeply concave. 2 long and 2 shorter setae near the end on the dorsal surface and 2 setae at the sides. A row of short setae at the posterior margin. Dorsal surface otherwise bare. Sternite 1 + 2 short, with a ctenidium of about 30 thick blunt teeth. There are 2 rows of short spines on the posterior part of the surface and some smaller spines anteriorly at the sides. Sternites 3 and 4 with slightly concave hindmargins and uniform marginal rows of thick setae which stand more closely at the sides. Surface covered with short setae. Sternite 5 trapezoid, as long as 3 and 4 together, with a row of long setae at the posterior margin which are shorter and thinner in the middle. The row of spines at the hindmargin which is characteristic for the subgenus *Cyclopodia* is absent. Surface covered with short spines anteriorly and longer setae posteriorly except for a bare area in the middle posteriorly in some specimens.

Genitalia (Figs. 53*a–c*). Claspers long and slender, parallel sided in the basal three-quarters and tapering to a short blunt tip in the apical quarter. A row of minute spines in the apical half and a long and 2–3 short setae near the base on the dorsal surface. A row of about 8–10 short thick spines in the apical quarter of the ventral surface. These spines are thick and curved near the tip and become gradually longer and thinner towards the base. Several rows of minute spines on the ventral surface. Basal arc triangular. Basal plate very long and narrow, wider

anteriorly, with a deep concavity anteriorly. Phallobase triangular with a bifid, slightly hooked tip and an irregular double row of spines along the outer margins up to the apical quarter. The parameres form the ventral part of the phallobase. They are not mobile and have a posterior process with curved spines. They have

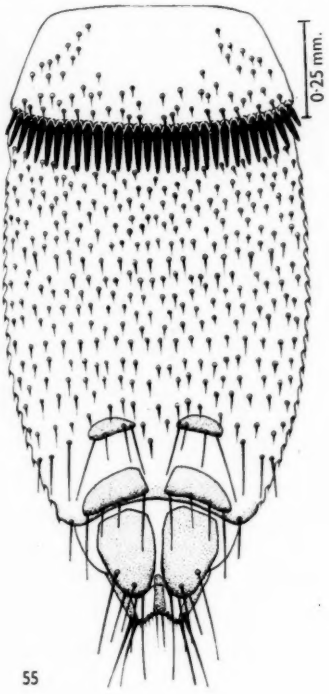


Figs. 51–54. *Cyclopodia ferrarii*. Fig. 51. Male, abdomen, dorsal. Fig. 52. Male, abdomen, ventral. *a*, clasper, ventral. Fig. 53. Male genitalia. *a*, dorsal, aedeagus retracted; *b*, dorsal, aedeagus extended; *c*, lateral, aedeagus extended. *t*, tendon from paramere to aedeagus. Fig. 54. Female, abdomen, dorsal.

small spines on the surface and a deep ventral concavity. The aedeagus is membranous in its greater part and is sclerotized in 2 lateral, curved arms which are able to spread sideways. They widen anteriorly and bear there some teeth ventrally which are directed backwards. The aedeagus is invaginated anteriorly in the resting position and acts apparently in a similar manner as the endophallus of *Eucampsi-*

poda when extended and inflated. It can be protruded for a short distance from the phallobase. This is restricted by a tendon covered with minute teeth which originates at the posterior part of the paramere and is inserted at the base of the aedeagus. Apodeme long and slender with a narrow end-plate.

Abdomen, female (Figs. 54, 55). Tergite 1 + 2 with a marginal row of setae which are longer than in the male and with a similar premarginal row. Dorsum of the



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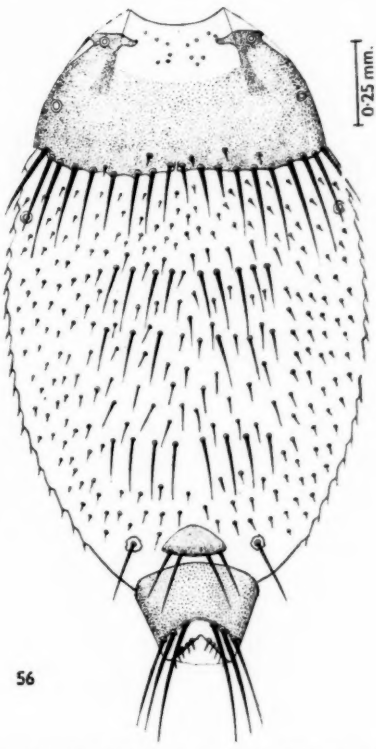

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Figs. 55, 56. Fig. 55. *Cyclopodia ferrarii*: female, abdomen, ventral Fig. 56. *C. ferrarii palawanensis* subsp.nov.: female, abdomen, dorsal.

abdomen covered with short spines. These are longer in some rows which may mark the posterior margin of the tergites. Such a row is best marked 3 rows anterior to tergite 6 and is probably the posterior margin of tergite 5. Tergite 6 is small, elliptical or triangular and bears 3–5 long setae at the posterior margin. Surface bare. Anal segment short, conical, with a row of long setae around the anal frame and 2 more premarginal long setae ventrally. Dorsal surface bare. Sternite 1 + 2 as in the male. Venter covered with short spines on sclerotized bases in the anterior two-thirds which are longer posteriorly. Sternite 5 has 2 small crescent-shaped sclerites which bear 2 long setae in each corner and 2 vertically standing setae in the middle of the hindmargin. Sternite 6 with 2 larger, triangular sclerites with a long seta at the lateral and median corners and 2 to 3 vertically standing setae on

the hindmargin. Sternite 7 consisting of two triangular sclerites which bear several long setae posteriorly and 1 or 2 on the surface. Anal sclerite narrow, strip-like, with 2 setae posteriorly. Sternite 7 functions as genital plate.

Distribution and hosts. Java (type locality); India, Ceylon, Burma, Siam, Malaya, Borneo, Sumatra. (See map no. 1, p. 306). Mainly on species of the genus *Cynopterus*, a few stragglers on insectivorous bats.

Material examined:

INDIA. Madras, *Cynopterus sphinx*, 1 ♂; Bellari, *Megaderma lyra*, 1 ♀; Calcutta, 2 spec.; Dacca, Bengal, *Cynopterus marginatus*, 3 spec. (Traub); Bombay, *C. sphinx*, 5 spec. (Hiregaudar).

CEYLON. Urugala, C.P., 15 spec. (Major); Peradenyia, 2 spec. (Pearson); *Cynopterus brachyotis ceylonensis*, 3 spec. (Fryer); Gonagama, Kitulgala, *C. brachyotis ceylonensis*, 1 ♂ (Phillips); Matugama, Kalutara, *C. sphinx*, 2 spec. (Phillips); Namunukala, *C. brachyotis ceylonensis*, 8 spec. (Phillips).

BURMA. Bhamo, 3 spec. (Fea); Kani, Lower Chindwin, 8 spec. (Aung Shwe).

SIAM. Bangkok, *Cynopterus sphinx*, 1 ♀ (Boden Kloss); Nakhon Pathom, Luang Lampaya, *C. brachyotis angulatus*, 5 spec. (Elbel); Rat Buri, Ban Pong, Tapah, *C. brach. angulatus*, 2 spec. (Elbel); Kwan Don, Satul prov. *Cynopterus* sp., 13 spec. (Sanborn).

MALAYA. S. Perak, *Cynopterus brachyotis angulatus*, 1 ♂ (Robson); Selangor prov., Ulu Langat, Kuala Lumpur, Kepong, Bukit Lagong Forest reserve. *Cynopterus* sp., *C. horsfieldi*, *Chironax melanocephalus*, *Megaerops ecaudatus*, *Myotis muricola*, 45 spec. (Traub, British Scrub Typhus Research Unit).

JAVA. Buitenzorg, *Cynopterus sphinx*, 10 spec. (Heurn); W. Java, *C. horsfieldi*, 1 ♀ (Balston and Shortridge).

SUMATRA. Krapoh Deli, Bedagri, *C. brachyotis angulatus*, 1 ♂ (Kanneguger); Balighe, W. Sumatra, 2 spec. (Modigliani).

BORNEO: Mt Kinabalu, Ranau, *Cynopterus* sp. 1 ♂ (Traub); Billiton Island, *C. brachyotis*, 1 ♀ (Abbott).

Schuurmans Stekhoven & Hardenberg (1938) give a number of localities in Java, Sumatra and Simalur Island and the following additional species of hosts: *Macroglossus minimus*, *Rousettus amplexicaudatus* and *Pipistrellus abramus*.

Ceylon specimens differ in some details in the male genitalia from the typical form, but these differences are rather variable and more material from India is needed before a subspecies can be established.

(19a) **Cyclopodia ferrarii palawanensis** subsp.nov.

The subspecies differs from the typical form in the presence of rows of longer setae in the posterior rows of the segments on the dorsum of the female. Such rows are well marked in the marginal row of tergites 3 and 5 (Fig. 56). In the typical form the short spines of the dorsum are longer posteriorly and the marginal row of tergite 5 may consist of spines longer than those of the surroundings, but there are no long setae. In the male, the phallobase and parameres are of different shape. The anterior hook of the phallobase is stouter and more strongly curved upwards.

The parameres are longer and less strongly concave ventrally. The row of spines on the ventral surface of the claspers consists of 10–12 spines which are all thick and blunt, but become smaller towards the base. In the typical form the more basal spines are much thinner and more pointed than the apical ones (Fig. 57).

Distribution and hosts. PHILIPPINES: Palawan Island, Brooke's point, Iwahig, Puerto Princessa; Busuanga and Culion Islands, north of Palawan Island, *Cynopterus brachyotis luzoniensis*, c. 115 spec. (Hoogstraal, Celestino, Werner), type series (Chicago Natural History Museum).

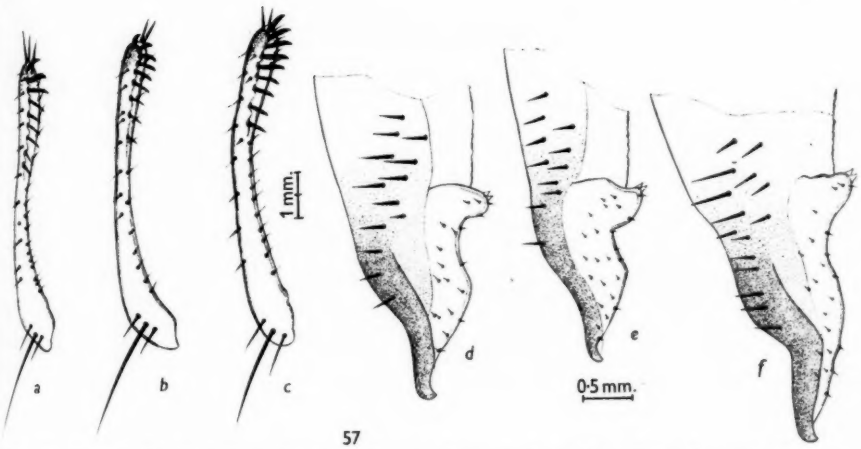

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Fig. 57. *Cyclopodia ferrarii*: male, genitalia. *a*, claspers of specimen from Java; *b*, of specimen from Ceylon; *c*, of *C. ferrarii palawanensis* subsp.nov.; *d*, phallobase, lateral of specimen from Java; *e*, of specimen from Ceylon; *f*, of *C. ferrarii palawanensis* subsp.nov.

(20) **Cyclopodia obliqua** sp.nov.

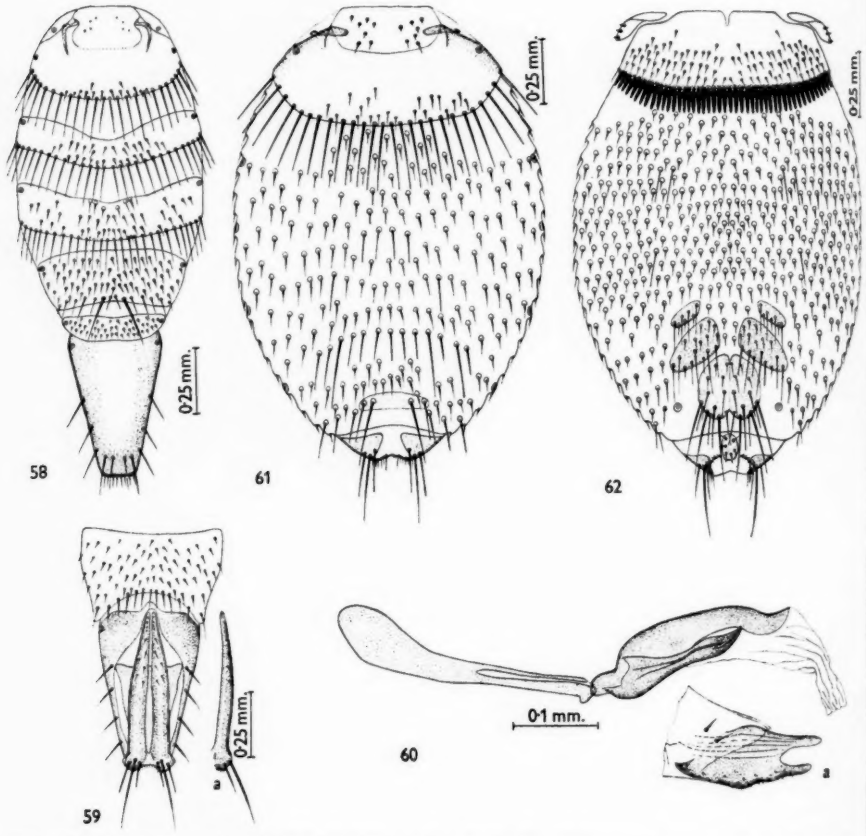
Length 2·5 mm. Colour yellowish brown.

Head. Vertex bare. 2 setae on the anterior dorsal margin. Eyes with 2 incompletely separated ocelli. A row of short seta along the anterior ventral margin and 2 premarginal rows on the genae. Palps with thick base and tapering towards the tip as in *Eucampsipoda*. They have a moderately long terminal seta and 2 shorter setae close to it. There are 5 to 6 setae along the ventral margin and 1 to 2 near the base of the dorsal edge. Labella of labium about half as long as the theca. 3 to 4 minute spines at the posterior margin in each half.

Thorax as long as wide, triangular, narrow between the ctenidia which have about 15 slender, blunt teeth. Oblique sutures forming an angle of about 80°. 4–5 notopleural setae in a row. Legs shorter than in *C. ferrarii* (2·4–2·6 mm).

Abdomen, male (Figs. 58, 59). Postspiracular sclerite with 1 to 3 short spines. Tergite 2 with a uniform marginal row of moderately long setae and a premarginal row of about 15 short spines. Tergites 3 and 4 shorter than in *C. ferrarii* and with shorter and more numerous spines on the surface. Tergite 5 longer, its surface

covered with short spines and with a marginal row of short setae and 2 long setae near the middle. Tergite 6 short and narrower than 5, without a marginal row and its surface covered with minute spines. Anal segment slender, conical, shorter than in *C. ferrarii*, its width at the base about two-thirds of its length. It has 3 setae of moderate length laterally in the posterior half, a transverse row of 4 such setae a short distance before the hindmargin and a row of about 8 short setae at the



Figs. 58–62. *Cyclopodia obliqua* sp.nov. Fig. 58. Male, abdomen, dorsal. Fig. 59. Male, abdomen, sternite 5 and genital area. *a*, clasper, lateral. Fig. 60. Male, genitalia, lateral. *a*, paramere. Fig. 61. Female, abdomen, dorsal. Fig. 62. Female, abdomen, ventral.

hindmargin. Abdominal ctenidium of 42 teeth. Sternite 5 narrower posteriorly, not much longer than 4, with concave hindmargin which carries a marginal row of short setae and a premarginal row of similar setae. There are about 4 rows of short setae on the surface.

Genitalia (Fig. 60). Claspers slender, tapering to a blunt tip in the apical half. One long and 2 shorter setae dorsally near the base. A row of minute hairs on the outer edge and some minute spines near the tip. 2–3 rows of short spines in the

apical half of the dorsal surface. The row of spines on the ventral surface which is characteristic for *C. ferrarii* is absent. Basal arc triangular. Basal plate also triangular but shorter and broader than in *C. ferrarii*. Phallobase with straight anterior point, fused with the basal arc. It bears 2 setae at each side. Parameres broad, with two long apical processes with a deep concavity between them and several rows of minute hairs near the ventral edge. Aedeagus sclerotized ventrally in 2 pointed lateral ridges and dorsally. It is membranous anteriorly and may possibly become inflated when extended.

Abdomen, female (Figs. 61, 62). Tergite 1 with 2 groups of 7 minute spines. Tergite 2 rounded posteriorly with a uniform marginal row of moderately long setae and a premarginal double row of about 15 short spines in the middle. Dorsum covered with spines which are short anteriorly and much longer posteriorly. There are 4 long setae in the marginal row of tergite 5 near the middle. Tergite 6 small, transversely elliptical, ill defined, carrying 2 long and 2 shorter setae. Anal segment very short, with 2–3 long setae posteriorly. Venter covered with short spines. There are 4 longer setae in the premarginal row of sternite 3 and 2 in the premarginal row of sternite 4. Sternite 5 curved, with 2 small, elliptical, obliquely standing sclerites which carry 2 rows of setae. Sternite 6 similar, but the sclerites are larger and stand nearer to the middle line. They bear a row of long setae in the middle and rows of shorter setae anteriorly and along the hindmargin. Sternite 7 consisting of 2 elliptical sclerites with 5–7 long setae posteriorly and shorter setae anteriorly. Anal sclerite small, elliptical, with 6 short setae.

Material examined. MALAYA: Kepong, *Balionycteris maculata*, 1 ♂, 1 ♀ (Traub), types (Chicago Natural History Museum).

(21) **Cyclopodia simulans** sp.nov.

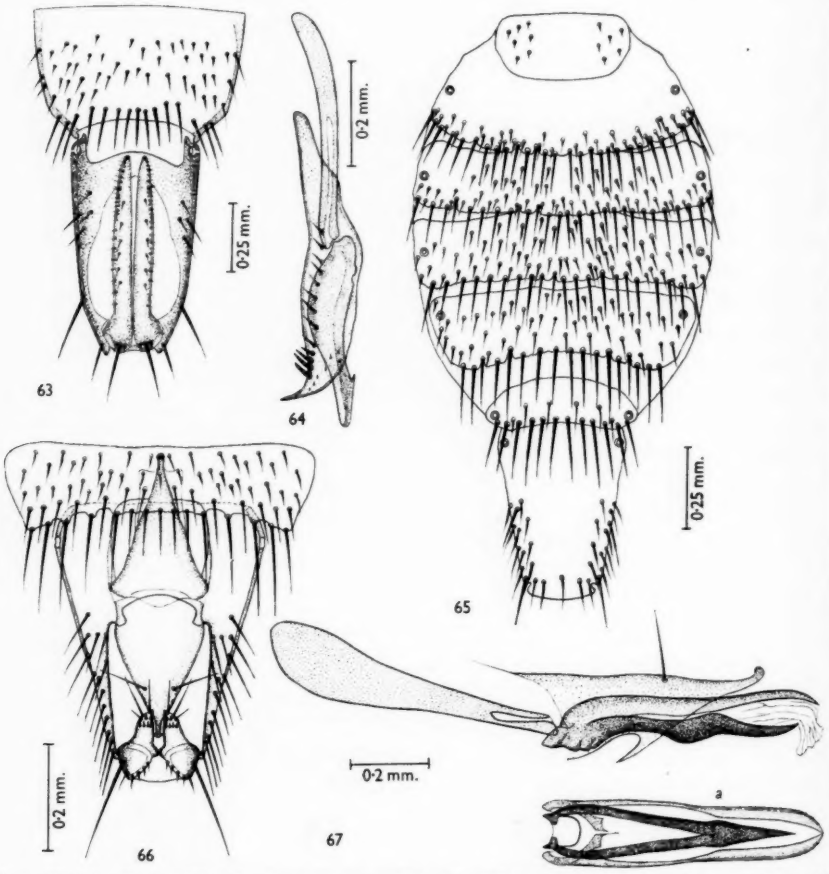
Length 3 mm. Colour yellowish brown.

Head. Vertex bare. 2 long setae at the anterior dorsal margin. Eyes with incompletely separated ocelli, with large lenses on a common, shallow, pigmented base (Fig. 50 B, *e*). Palps with a thick base, tapering towards the tip, with a moderately long terminal seta, and 4–5 short setae along the edges. Labella of labium slightly longer than the theca. 3 minute spines at the posterior margin of the theca in each half.

Thorax slightly wider than long. 2 notopleural setae. Oblique sutures forming an angle of 90°. Otherwise very much as in *C. ferrarii*. Legs shorter than in *C. ferrarii*. Leg 1 = 2·2 mm., legs 2 and 3 = 2·4 mm. long. Metatarsi of all 3 legs of about equal length (0·55–0·58 mm.) (0·65–0·85 mm. in *C. ferrarii*).

Abdomen, male (Fig. 63). Very similar to that of *C. ferrarii*. Postspiracular sclerite without spines. There is a double row of about 18 short spines on the surface of tergite 2 near the marginal row. Anal segment slender, shorter than in *C. ferrarii*, tapering slightly, twice as long as wide at the base. Some short setae on the lateral posterior part of the dorsal surface, a transverse row of 4 longer setae a short distance before the hindmargin and a row of 8 short setae at the hindmargin.

Genitalia (Fig. 64). Claspers slender, tapering to a blunt point. A long and 2 short setae dorsally near the base. A row of minute hairs along the outer edge and a row of short spines on the dorsal surface near the outer edge. The row of spines on the ventral surface which is characteristic for *C. ferrarii* is absent. Phallobase bifid at the tip, with upturned ends and a row of spines which are



Figs. 63–67. *Cyclopodia simulans* sp.nov. Fig. 63. Male, abdomen, sternite 5 and genital area. Fig. 64. Male, genitalia, lateral. Fig. 65. *C. brachythrinax* sp.nov.: male, abdomen, dorsal. Fig. 66. Male, abdomen, sternite 5 and genital area. Fig. 67. Male, genitalia, lateral. *a*, aedeagus, ventral.

thicker anteriorly along the outer edge of the base. Basal arc triangular, fused with the phallobase. Parameres fused with the phallobase, with 2 rows of minute spines. Aedeagus wholly sclerotized, curved, tapering to a rounded point and with a small ventral tooth near the tip. Apodeme slender, as long as the aedeagus.

Female unknown.

Material examined. PHILIPPINES: Todaya, Mindanao, Mt Apo, *Ptenochirus jagori*, 2 ♂♂ (Alcasid), types (Chicago Natural History Museum).

This species resembles *C. ferrarii* closely in chaetotaxy and structure of the body but differs in the form of the claspers and the genitalia.

(22) **Cyclopodia brachythrinax** sp.nov.

Length 2·5 mm. Colour yellowish brown.

Head. Vertex bare. 4 setae at the anterior dorsal margin. Eyes with incompletely divided ocelli on a common base, but more deeply divided than in Basilia (Fig. 50B, *d*). Palps with a moderately long terminal seta. 2 shorter setae near the tip, 1 in the middle of the dorsal surface and 4–5 at the ventral edge. A row of short setae along the anterior ventral margin of the head and 2–3 rows on the genae. Labella of the labium slightly longer than the theca. 3 minute spines at the posterior margin of the theca in each half.

Thorax slightly wider than long, rounded anteriorly. Ctenidium with about 16 blunt teeth. One longer and one shorter notopleural seta. Sternal plate covered with short spines. A dense row of short spines near the posterior margin with 3 longer and more widely spaced setae at each side. A patch of minute hairs near the posterior end of the oblique sutures and anteriorly between the ctenidia, but no such minute hairs scattered between the short spines. Legs short, as in *C. simulans* (2·4–2·5 mm. long). All 3 legs of about equal length.

Abdomen, male (Figs. 65, 66). Postspiracular sclerite with 1–2 short spines. 2 groups of 7 minute spines on tergite 1. Tergite 2 with a marginal row of moderately long setae and a double premarginal row of short setae on the surface. Tergites 3–5 short, of about equal length, with marginal rows of moderately long setae and short setae on the surface. The lateral marginal setae of tergite 5 longer. Tergite 6 shorter and narrower, with a (?) similar marginal row (setae missing) and a transverse row of 4–5 short setae on the surface. Anal segment slender, tapering, slightly less than twice as long as wide at the base. Some short setae at the lateral posterior parts of the dorsal surface and a few short setae at the posterior margin. Sternite 1 + 2 with a ctenidium of 42 thick spines. 3–4 rows of short spines in the posterior part of the surface. Sternites 3–5 about equal, short, with moderately long marginal setae. Those of sternite 5 longer at the sides. Surface covered with short spines.

Genitalia (Fig. 67). Claspers rudimentary, very short, with 2 longer setae at the tip and a few short spines further basally. A long seta dorsally near the base. Basal arc triangular, with an apical knob. Phallobase also with apical knob and 2 setae at the sides. Aedeagus long, sclerotized ventrally and dorsally at the sides and membranous in the middle of the dorsal surface and at the sides. The ventral part is narrowly triangular in dorsal view with a wavy lateral outline. The dorsal ridges are more weakly sclerotized and end in 2 points. Apodeme as long as the aedeagus with a moderately broad end-plate.

Female unknown.

Material examined. NORTH BORNEO: Mt Kinabalu, Paring rest house, 1 ♂, type. (Phang Ong Wah) (Chicago Natural History Museum).

(23) **Cyclopodia macrura** Speiser, 1901

No further records of this species have been published since Speiser's original description.

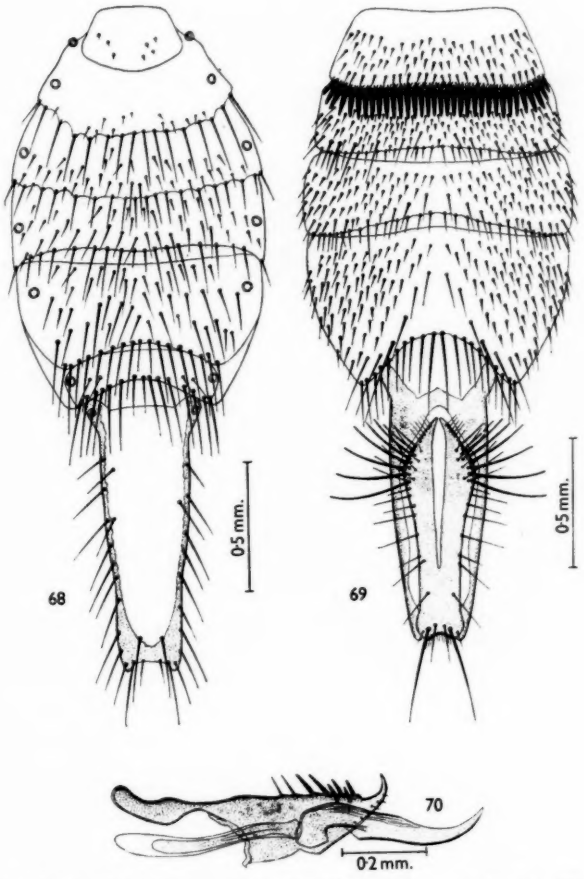
Length: female 3·5–4 mm., male 4·5–5 mm. of which the anal segment is 1·25–1·5 mm. Colour brown.

Head. 4–5 setae at the anterior dorsal margin. Vertex bare. 5–6 short setae at the anterior ventral margin. 1 seta in the middle of the row is longer than the others. Genae bare. Eyes with deeply, but not completely separated ocelli with large prominent lenses (Fig. 50 B, *f*). Palps long and slender, tapering towards the tip, with a long terminal seta and 4–5 setae at the dorsal and ventral margins which are longer towards the tip. A long seta in the middle of the external surface. Labella of the labium nearly twice as long as the theca. 3 minute spines at the posterior margin of the theca in each half.

Thorax slightly wider than long. Angle of the oblique sutures about 90°. Area behind the oblique sutures covered with rather long spines. Anterior to the oblique sutures there is a row of shorter spines along the sutures and the area between the ctenidia is covered with minute hairs. There is a patch of such minute hairs also near the posterior end of the oblique sutures. Thoracic ctenidium with about 12 very thick, blunt teeth. Haltere groove open. 2–3 notopleural setae. Legs long. Anterior coxae cone-shaped, two-thirds of the length of the femur. Tibiae cylindrical, slightly compressed laterally, 5–6 times as long as wide. 3 rings in the middle of the tibiae, of which the apical ring is farther away from the middle one than the basal ring. Tarsi about as long as the tibiae.

Abdomen, male (Figs. 68, 69). 2 groups of 3–4 minute spines each on tergite 1. Tergite 2 broadly rounded with a continuous marginal row of widely spaced, thick, moderately long setae. A premarginal row of short spines in the middle. Tergites 3 and 4 with similar marginal rows and short spines on the surface. A few premarginal longer setae on tergite 4. Tergite 5 as long as 3 and 4 together, narrower posteriorly with a marginal row of longer setae and with longer setae in the posterior part and shorter ones in the anterior part of the surface. Tergite 6 very short, strip-like, with a concave posterior margin which bears 15–16 long setae. Surface with only 3–4 short setae or bare. Anal segment very long and slender, nearly cylindrical, about 3 times as long as wide at the base in mounted specimens, about 4 times as long in alcohol specimens. Dorsal surface bare, except for 2 long setae near the posterior margin and a few others near the sides in the posterior half. 2 long setae and a few shorter ones at the posterior margin and some shorter setae along the sides of the segment. Sternite 1 + 2 with a ctenidium of about 30 blunt, thick teeth. Surface with 3–4 rows of long spines in the posterior and lateral parts. Sternites 3 and 4 with marginal rows of spines. Surface covered with short spines and a premarginal row of longer setae. Sternite 5 as long as 3 and 4 together, narrowing posteriorly, with a deeply concave posterior margin which bears a row of setae which are longest in the middle. There is a V-shaped row of moderately long setae in the posterior part of the surface. The area inside the V is bare and the anterior lateral parts of the surface are covered with short spines.

Genitalia (Fig. 70). Claspers fused at the base, nearly parallel sided in the basal two-thirds, widening in the apical third and tapering to a point. The lateral margin of the wide apical parts bears a row of about 8 long, blunt spines or setae which become gradually shorter towards the tip. The longest, basal seta stands at about

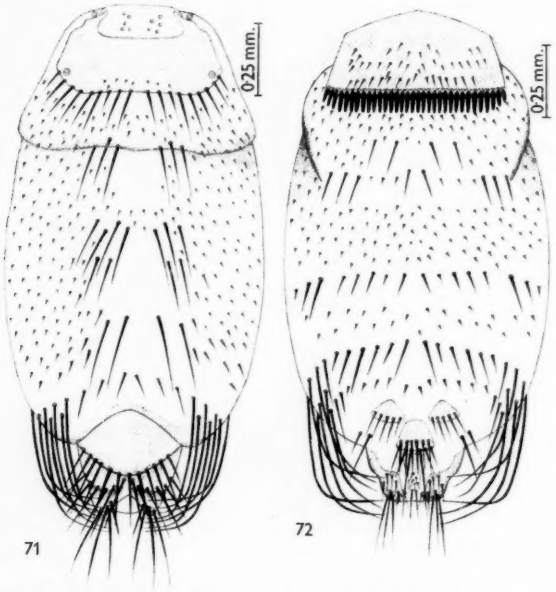


Figs. 68–70. *Cyclopodia macrura*. Fig. 68. Male, abdomen, dorsal. Fig. 69. Male, abdomen, ventral. Fig. 70. Male, genitalia, lateral.

the apical quarter. 2 long and 4 short setae dorsally near the base and short setae in the basal half and along the sides. Some fine hairs at the lateral margin near the tip and a row of such hairs on the ventral surface also near the tip. Basal arc with long, rounded anterior process, fused with the phallobase. Basal plate long, rectangular, with a deep anterior concavity and a short process posteriorly. Phallobase triangular, bifid apically and terminating in 2 curved points which are directed upwards and inwards. There is a double row of about 8 long, thick spines in each half of the phallobase which are longest near the tip. Aedeagus sclerotized

throughout, broad basally, tapering to a fine point, curved, with a deep indentation ventrally near the base. Apodeme slender, about as long as the aedeagus, with a narrow end-plate.

Abdomen, female (Figs. 71, 72). Tergite 1 + 2 longer than in the male and with a row of setae which are longer laterally at the posterior margin. There is a gap in the middle of the row. Surface bare except for a premarginal row of 6–8 short spines. Dorsum of the abdomen membranous with 3 groups of setae in each half which apparently indicate the posterior margins of the tergites. The hindmargin of tergite 3 is further marked by a fold of the integument which was considered by



Figs. 71, 72. *Cyclopodia macrura*. Fig. 71. Female, abdomen, dorsal. Fig. 72. Female, abdomen, ventral.

Speiser as the hindmargin of the basal segment. The dorsum is covered with short spines except in some median areas between the groups of long setae. There are 2 groups of 3–6 setae in one or 2 rows a short distance behind tergite 2 which mark the hindmargin of tergite 3. Two further groups of 6–8 setae in 2–3 rows in the middle of the dorsum at the hindmargin of tergite 4. The posterior margin of tergite 5 is indicated by a transverse row of very long setae laterally and shorter setae towards the middle. The long lateral setae are curved towards the anal segment. There is a transverse row of long setae, which is interrupted in the middle, on the surface of tergite 5. Tergite 6 with a triangular sclerite in the middle which is bare and bears a row of long setae at the posterior margin. There are 2–3 rows of long curved setae at the sides of the surface of the tergite in a specimen of the type series and only a single row in the specimen from Sumbawa. These

curved setae, together with the lateral marginal setae of sternite 5, form a thick brush. Anal segment short, truncate, with a curved row of long setae on the dorsal surface around the anal frame and a group of long setae at the ventral anal processes. Sternite 1 + 2 with a ctenidium of 35 teeth and short spines, mainly in the middle of the posterior part of the surface. Sternite 3 with 4–5 rows of short spines on the surface and a row of moderately long setae at the hindmargin. 4 long setae in the middle of the premarginal row. Sternite 4 similar but there are 2 long setae laterally in the marginal row. Sternite 5 with a marginal row of long setae and 2 rows of short setae on the surface. Sternite 6 with 2 small triangular sclerites in the middle which have a row of long setae posteriorly. A few long setae continue these rows laterally in one specimen. A single row of short setae on the surface anterior to the sclerites. Sternite 7 with a square or trapezoid sclerite in the middle which bears 2 long setae in the middle of the marginal row and shorter setae laterally. A premarginal row of shorter setae. Anal sclerite triangular with 2 long setae posteriorly and some short setae on the surface near them.

The description of the female is based on a specimen of the type series and 2 specimens from other localities. There are a number of differences in the chaetotaxy of these specimens and it has to be left open for the moment whether these differences are within the range of variation of the species or perhaps of subspecific rank.

Material examined. New Britain, *Dobsonia peroni*, 1 ♂, 1 ♀, type series (Dahl); Sumbawa: Wawo, 1 ♀, 1 ♂. New Guinea, Bulolo, 1 ♀ (Rio).

(24) **Cyclopodia orthotricha** sp.nov.

This species is closely related to *C. macrura*, but differs in its smaller size and a number of chaetotactic characters. When more information about the variability and distribution of *C. macrura* is available, this species may have to be considered as a subspecies of *C. macrura*. These specimens were determined as 'a small form of *C. macrura* differing from the typical form in minute points of chaetotaxy' by Scott and published by Thompson (1937).

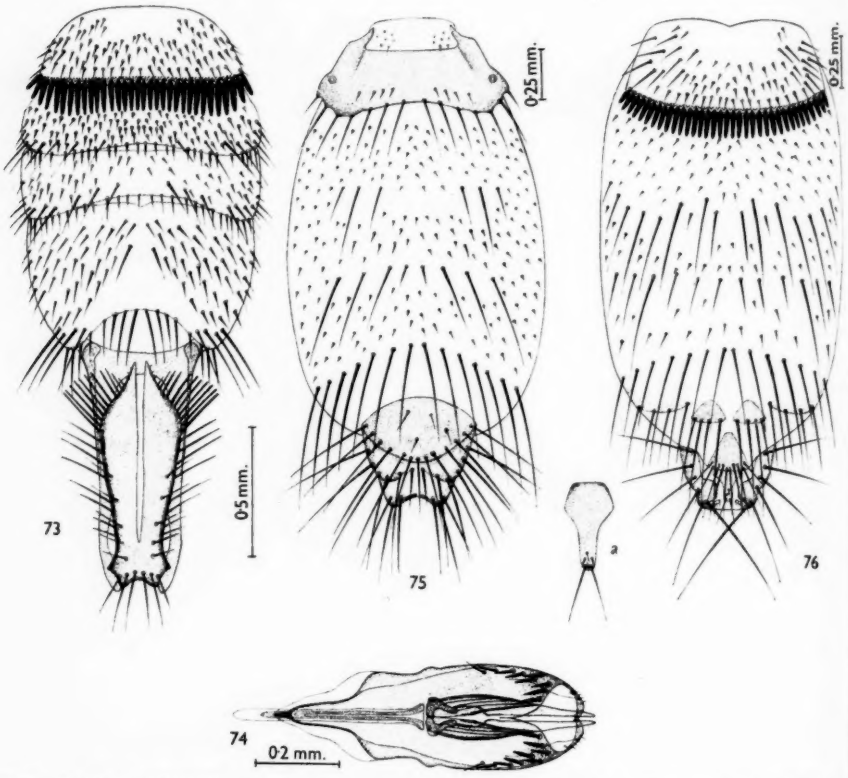
Description. Length of female, 3–3·5 mm. Male: 3·5 mm. of which the anal segment is 1 mm.

Head as in *C. macrura*. The anterior ventral margin is more strongly curved than in *C. macrura*, forming nearly a right angle. *Thorax* and legs as in *C. macrura*.

Abdomen, male (Fig. 73, 74). Only the differences from *C. macrura* will be noted. The posterior spines of the groups on tergite 1 are much longer than the anterior ones and there are about 8 spines in each group. The setae on the tergites are more numerous. Tergite 6 is bare on the surface and has only 12 marginal setae as against 16 in *C. macrura*. The anal segment is shorter, only twice as long as wide at the base in mounted specimens and 3 times as long in alcohol specimens. The claspers are wider near the tip and widen gradually from the base. The setae near the tip are shorter and have truncated tips. The basal seta stands at the apical third of the clasper.

Abdomen, female (Figs. 75, 76). The chaetotaxy differs markedly from that of

C. macrura. The whole dorsal surface is covered with spines without any median bare areas. The long setae are arranged in 3 transverse rows, indicating the posterior margins of the tergites. There are only 2–3 long setae in the marginal row of tergite 3 and 6 in the marginal row of tergite 4. The marginal row of tergite 5 consists of long setae which extend across the abdomen on to the pleurae, but the lateral setae are straight, not curved as in *C. macrura*. The median setae of the row are as long as the lateral ones while in *C. macrura* the median setae are much shorter. The surface of tergite 6 is bare, except for a few short spines on the sclerite.



Figs. 73–76. *Cyclopodia orthotricha* sp.nov. Fig. 73. Male, abdomen, ventral. Fig. 74. Male, genitalia, dorsal. Fig. 75. Female, abdomen, dorsal. Fig. 76. Female, abdomen, ventral. *a*, anal sclerite.

The curved setae at the sides of tergite 6 which are characteristic for *C. macrura* are absent. The row of short setae on the surface of sternite 6 anterior to the sclerites is also absent. Sternite 7 is triangular and has only a single irregular row of setae posteriorly and 2 additional setae at the corners instead of the 2 rows of *C. macrura*.

Material examined. SOLOMON ISLANDS: Fatura, Isabel Island, *Dobsonia inermis*, 2 ♂, 4 ♀ (Lever). Type series, British Museum (Nat. Hist.).

KEY TO THE SPECIES OF THE GENUS *CYCLOPODIA*

Males of the species of the subgenus *Cyclopodia* have very few characters useful for identification. Particularly the males of the *sykesii* group are all very similar and difficult to distinguish. The males of *C. sykesii* and *C. horsfieldi* are indistinguishable by any character, including those of the genitalia. The aedeagus rod and the extent of the field of spines of the connecting tube are useful in some species (Fig. 77). They are clearly visible through the body wall in cleared preparations. The form of the phallobase and the arrangement of the setae on its base are also useful in some instances. In addition to this uniformity, the great variability of the species makes the chaetotactic characters even more unreliable. The form of the claspers and their thickness, i.e. the relation between the length and thickness at the base varies to such an extent that it is practically useless, except in a few instances.

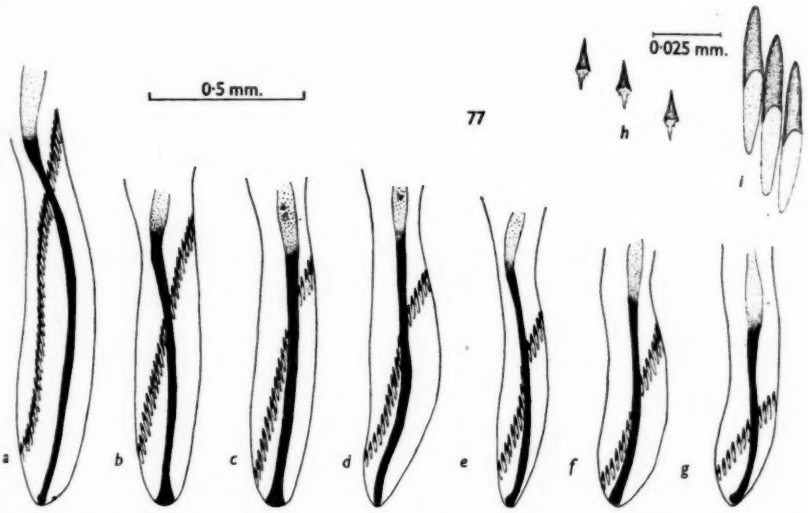


Fig. 77. Genitalia of species of the *sykesii* group (semi-diagrammatic). The aedeagus rod, the distal border of the field of spines of the connecting membrane and the outline of the invaginated connecting membrane only are shown. *a*, *Cyclopodia sykesii*; *b*, *C. albertisi*; *c*, *C. similis*; *d*, *C. oxycephala*; *e*, *C. bougainvillensis*; *f*, *C. ponapensis*; *g*, *C. inclita*; *h*, teeth of the connecting membrane of *C. australis*; *i*, teeth of the connecting membrane of other species of the *sykesii* group.

The females are easier to identify. The long setae on the abdomen give good characters for the separation of the species, but here again, the great variability of the species has to be taken into account. The form of the genital plate affords specific characters in some instances.

Males

1. Anal segment broadly conical, less than twice as long as wide at the base. Tergites 5 and 6 much shorter than 4. A row of thick, short spines at the hindmargin of sternite 5. Genitalia with a membranous aedeagus without apodeme.
(Subgenus **Cyclopodia** s.str.) 2

— Anal segment slender, nearly cylindrical, twice as long as wide at the base or longer. Tergite 5 as long as, or longer than, 4. A row of spines at the hindmargin of sternite 5 is absent. Aedeagus partly or wholly sclerotized and with an apodeme.
(Subgenus **Leptocyclopodia** subg.nov.) 15

2. Haltere groove completely closed by a cover. Marginal rows of some or all abdominal tergites interrupted in the middle. (Exception *C. australis* sp.nov.) Oriental and Pacific Region (Figs. 3*a*, 6). (**sykesii** group) 3

— Haltere groove either partly closed or open. Marginal rows of the abdominal tergites continuous (Figs. 3*b*, *c*, 25, 34). 8

3. Cover of the haltere groove attached along its whole length. Only marginal row of tergites 2 and 3 interrupted in the middle. Only 6 spines at the hindmargin of sternite 5. Vertex bare anteriorly, but with some setae in its posterior part (Figs. 20–22).
Cyclopodia minor Speiser

— Cover of haltere groove attached only in its posterior third. Vertex with setae on the anterior part of the surface. 4

4. Notopleural setae absent. Marginal rows of abdominal tergites consisting of short spines only on tergites 2 and 3, of a group of setae in the middle third on tergite 4 and continuous rows on tergites 5 and 6. Only 8–10 hairs on the vertex between the eyes. Spines on the connecting membrane of the aedeagus short and triangular (Figs. 15, 77*h*). **Cyclopodia australis** sp.nov.

— Notopleural setae present. Marginal rows on abdominal tergites 2–6 broadly interrupted in the middle. Spines of connecting membrane of aedeagus long and narrow (Fig. 77*i*). 5

5. Vertex covered with setae except in a posterior narrow strip. Most setae at the posterior lateral corners of tergite 2 longer than half the length of the marginal setae of tergite 3. Hook of phallobase slender. 6

— Only the anterior half or less of the vertex covered with setae. Most setae at the posterior lateral corners of tergite 2 shorter. Hook of phallobase broad and short. 7

6. Aedeagus rod 1·3–1·5 mm. long. Field of spines of the connecting tube as long as the rod. 20–30 minute spines on tergite 1, the posterior spines of the group much longer than the anterior ones (Fig. 77*a*).
Cyclopodia sykesii Westwood, **C. horsfieldi** de Meijere

— Aedeagus rod 0·85 mm. long. Only 18–24 minute spines on tergite 1. The posterior ones not much longer than the anterior spines. **Cyclopodia similis** Speiser

7. A triangular group of about 20–30 spines on the anterior half of the vertex. Aedeagus rod 0·72–0·85 mm. long. Field of spines of the connecting membrane as long as the rod or nearly so (Fig. 12). **Cyclopodia albertisi** Rondani, **C. ponapensis** sp.nov.

— Less than half the vertex covered with about 20–25 setae. Aedeagus rod 0·8 to 0·9 mm. long, covered only in two-thirds or three-quarters of its length by the field of spines. **Cyclopodia oxycephala** Bigot, **C. bougainvillensis** sp.nov.

— Vertex as above, but with only 12–15 setae. Aedeagus rod 0·6–0·7 mm. long, covered only in half its length by the field of spines (Fig. 17). **Cyclopodia inclita** Falcoz

8. Haltere groove partly closed by a flap which is attached to the notopleural suture along its whole length and by a large rounded bulge opposite. Eyes small (Fig. 3*b*).
(**pembertoni** group) 9

— Haltere group open (Fig. 3*c*). **10**

9. Vertex and anterior margin bare. 2 notopleural setae. About 10 short, thick club-shaped spines on sternite 5. Claspers thick, 4 times as long as wide at the base.
Cyclopodia pembertoni Scott

— A row of about 10 setae at the anterior dorsal margin of the head and a double row of about 16 short setae between the eyes. 7–8 notopleural setae in a group. Marginal rows of tergites 2–4 consisting of short spines, those of tergites 5 and 6 longer. A row of about 18 very long, truncate setae on sternite 5. Claspers slender, about 6 times as long as wide at the base. Hook of phallobase spatulate (Figs. 28–31).
Cyclopodia macracantha sp.nov.

10. Large species, 3·5–4·5 mm. long. Ethiopian Region and Madagascar. (**greeffi** group) **11**

— Small species, 2–3 mm. long. Oriental and Pacific Region. (**tenuis** group) **12**

11. 2 setae at the anterior dorsal margin of the head. 8–10 short, thick spines on sternite 5. Claspers slender, 6·5 times as long as wide at the base. Aedeagus rod long (1·2 mm.), curved, tapering to a point. Hook of phallobase long. Ethiopian Region (Fig. 35).
Cyclopodia greeffi Karsch

— Anterior dorsal margin of the head bare. About 20 spines at the hindmargin of sternite 5. Claspers thick and short, 3 times as long as wide at the base, with a group of spines at the rounded tip. Aedeagus rod short (0·5 mm.). Madagascar (Fig. 39).
Cyclopodia dubia Westwood

12. Length 3 mm. A truncate process with 8 short, club-shaped spines at the hindmargin of sternite 5. Aedeagus rod 0·6 mm. long. Phallobase with V-shaped double row of about 16 setae. Hook of phallobase short, pointed. Basal arc triangular with rounded anterior process (Figs. 46*d*, 49). **Cyclopodia truncata** sp.nov.

— Length 2–2·5 mm. Posterior margin of sternite 5 convex, but without truncate process. Aedeagus rod shorter, 0·33–0·45 mm. long. **13**

13. Vertex with a row of about 8 short setae between the eyes some of which stand at the margin. About 12 minute spines on tergite 1. Tergite 2 with a marginal row of short setae laterally and short spines in the middle. Surface covered with short spines in the middle and short setae at the sides. Numerous short spines on the surface of tergites 3 and 4. Basal arc broadly triangular with rounded anterior process. Aedeagus rod 0·33 mm. long. Phallobase with long hook and 6 setae at the base (Fig. 46*b*). **Cyclopodia solomonarum** sp.nov.

— Only 2 setae at the anterior dorsal margin of the head, vertex bare. The marginal row of tergite 2 consists of short setae of uniform length. **14**

14. About 10 rather long spines on tergite 1. Basal arc narrowly triangular, with pointed anterior process. Aedeagus rod 0·33 mm. long. Apical hook of phallobase long (Fig. 46*a*). **Cyclopodia tenuis** Schuurmans Stekhoven & Hardenberg

— 10–15 minute spines on tergite 1. Basal arc very broad basally with rounded shoulders. Aedeagus rod 0·45 mm. long. Phallobase with short pointed apical process (Fig. 46*c*) **Cyclopodia (?) inflatipes** Speiser

15. Small species (2·5–3 mm.). Labella of labium as long as the theca or slightly longer or shorter. Claspers not fused at the base. **16**

— Larger species (3·5–5 mm.). Labella of labium nearly twice as long as the theca. Claspers fused at the base, with triangular end which bears a row of long setae laterally. Aedeagus wholly sclerotized, curved, tapering to a point. **19**

16. 4 setae at the anterior dorsal margin of the head. Tergite 5 and sternite 5 not longer than 4. Claspers rudimentary, one quarter the length of the anal segment (Figs. 65–67). **Cyclopodia brachythrinax** sp.nov.

— 2 setae at the anterior dorsal margin of the head. Tergite and sternite 5 markedly longer than 4. Claspers long and slender. **17**

17. Claspers with a row of thick spines on the ventral surface near the tip. Anal segment 2–3 times as long as thick at the base. Aedeagus broad, membranous with 2 lateral sclerotized arms which bear teeth ventrally near the end (Figs. 50–53, 57).
Cyclopodia ferrarii Rondani

— Claspers without such a row of spines near the tip. Anal segment shorter. Aedeagus of different form. 18

18. Aedeagus wholly sclerotized, curved, tapering to a rounded tip and with a ventral tooth. Tergite 6 with a marginal row of setae and bare on the surface. Philippines (Figs. 63, 64). **Cyclopodia simulans** sp.nov.

— Aedeagus sclerotized ventrally in 2 ridges and dorsally, membranous at the sides. Tergite 6 without marginal row of setae and covered with minute spines on the surface. Malaya (Figs. 58–60). **Cyclopodia obliqua** sp.nov.

19. Length 4·5–5 mm. Anal segment 3–4 times as long as wide at the base. 7–8 minute spines of about equal length on tergite 1. Tergite 6 with 16 marginal setae. Claspers parallel-sided in the basal two-thirds. Basal seta of the apical row at the apical quarter of the clasper. Setae long, with blunt ends (Figs. 68–70).
Cyclopodia macrura Speiser

— Length 3·5 mm. Anal segment shorter, only 2–3 times as long as wide at the base. The posterior spines of the group on tergite 1 much longer than the anterior ones. Tergite 6 with 12 marginal setae. Claspers widening gradually towards the tip. The basal seta of the apical row at the apical third of the clasper. Setae short, with truncated ends (Figs. 73–74). **Cyclopodia orthotricha** sp.nov.

Females

1. No sclerites on abdomen between tergite 1+2 and anal segment and between sternite 1+2 and genital plate. (Subgenus **Cyclopodia** s.str.) 2

— Tergite 6 and sternite 5–7 or 6–7 bearing sclerites. Sternite 7 not transformed into genital plate. (Subgenus **Leptocyclopodia** subg.nov.) 19

2. Haltere groove completely closed by cover. Large species. Oriental and Pacific Region (Fig. 3*a*). (**sykesii** group) 3

— Haltere groove partly closed or open (Figs. 3*b*, *c*,). 13

3. Dorsum of abdomen covered with small spines on sclerotized bases only, without any groups of long setae or a field of large spines. 4

— Groups of long setae and of large spines on the dorsum of the abdomen. 5

4. Some setae at the anterior dorsal margin of the head and 25–30 short setae on the anterior half of the vertex. Pleurae covered with minute spines. No longer setae on pleurae or venter. Genital plate broader than long, with about 10 spines at the posterior margin in each half and a broad membranous area between the lateral sclerites which bear small spines. Cover of the haltere groove attached in its posterior part only (Fig. 16). **Cyclopodia oxycephala** Bigot

— Some longer setae on the anterior part of the pleurae and a transverse row of longer setae which is interrupted in the middle on the venter. Head with setae only at the anterior dorsal margin and on the posterior half of the vertex. Haltere groove cover attached along its whole length. Genital plate oblong, with about 6 spines at the hindmargin in each half (Figs. 20–21). **Cyclopodia minor** Speiser

5. Notopleural setae absent. Only 8–10 hairs on the vertex between the eyes. No group of large spines in the middle of the dorsum of the abdomen. 2 groups of 9–11 long setae each in the lateral posterior corners of the dorsum. **Cyclopodia australis** sp.nov.

— Notopleural setae present. 6

6. A single group consisting of long setae only or consisting of long setae and spines on the dorsum of the abdomen. 7

— A group of large spines on a bare area in the anterior part of the dorsum and one or more groups of long setae on the posterior part of the dorsum. 9

7. A single group of 20–30 long setae in transverse rows of 3–6 on the dorsum. In some specimens a few large spines in the anterior rows of the group, but no separate group of spines on a bare area. About 12–15 rows of minute spines anterior to the group of setae and 3–4 rows behind it (Fig. 19). **Cyclopodia inclita** Falcoz

– A smaller number of long setae in the dorsal group. **8**

8. The dorsal group is oblong and consists of 5–6 very long and strong spines anteriorly and 8–10 long setae posteriorly (Fig. 24). **Cyclopodia ponapensis** sp.nov.

– The dorsal group consists of 10–15 setae only and is situated near the posterior border of the spinose area. There are about 20 rows of minute spines in front of it and 3–4 behind it (Fig. 23). **Cyclopodia bougainvillensis** sp.nov.

9. The anterior group of spines is ill-defined and consists of 1–4 spines which are not much larger than some of the small spines surrounding. The group is not always in a bare area and is absent in some specimens (see no. 8). A group of 10–15 long setae near the posterior margin of the spinose area (Fig. 23).
Cyclopodia bougainvillensis sp.nov.

– The anterior group consists of 2–6 large spines on a bare area. **10**

10. A single, large group of long setae in the posterior part of the dorsum. No minute spines behind this group. Genital plate H-shaped. **11**

– 2 or 3 groups of long setae behind the anterior group of large spines. Genital plate without median bridge. **12**

11. About 6 rows of very long setae in the posterior third of the dorsum. This group is separated from the anterior group of large spines by several rows of minute spines. Only short spines on the pleurae (Fig. 9). **Cyclopodia sykesi!** Westwood

– Only 3–4 rows of long setae in the posterior third of the dorsum. This group extends anteriorly in a median stripe which may or may not reach the anterior field of large spines. A group of longer setae on the pleurae anterior to spiracle 4 (Fig. 10).
Cyclopodia horsfieldi de Meijere

12. 2 groups of long setae, each consisting of 2–7 setae, at the lateral posterior corners of the dorsum between spiracles 5 and 6. Only minute spines between these groups and the anterior group of 2–6 large spines. Genital plate square, with a median membranous stripe which is narrower in the middle (Fig. 14).
Cyclopodia albertisi Rondani

– 3 groups of setae on the dorsum. The 2 groups of long setae at the lateral posterior corners of the dorsum consist of 6–10 setae in most specimens (only 3 in one specimen) and are connected by a row of 4–8 setae in front of the anal segment. (This row is absent in the above specimen with 3 setae laterally). The anterior field of large spines continues posteriorly in a group of 7–10 long setae which, however, is separated from the posterior groups by a broad area of minute spines (Fig. 11).
Cyclopodia similis Speiser

13. Haltere groove partly closed by a flap which is attached to the notopleural suture along its whole length and a lateral, rounded bulge opposite. Eyes small. **14**

– Haltere groove open. **15**

14. Vertex bare. Abdomen very broad, trapezoid in dorsal view. Dorsum covered with spines anteriorly and setae posteriorly. Genital plate square, wholly sclerotized, with a long, curved horn at its base (Figs. 26, 27). **Cyclopodia pembertoni** Scott

– About 10 setae at the anterior dorsal margin of the head. A crescent-shaped group of about 50 long setae in the middle of the dorsum. In some specimens the median setae may be replaced by very long and strong spines. A row of about 8 long setae in front of the anal segment. Genital plate shield shaped and covered with short spines (Figs. 32, 33). **Cyclopodia macracantha** sp.nov.

15. Large species (3·5–5 mm). Ethiopian and Madagascar region. Some long setae on the abdomen. (**greeffi** group) **16**

— Small species (2–3 mm.). Oriental and Pacific region. No long setae on the abdomen. (**tenuis** group) 17

16. Anterior dorsal margin of the head bare. Dorsum of abdomen plane, covered with short spines which are minute in a broad median stripe and longer laterally and posteriorly in 2–3 rows. 2 groups of long setae, each consisting of 2 rows at the posterior border of the pleurae. Genital plate very small, triangular, with 6–8 spines which are longer than the plate. Madagascar (Figs. 41, 42).
Cyclopodia dubia Westwood

— 2 setae at the anterior dorsal margin of the head. Dorsum of abdomen forming a pronounced angle. Posterior part of dorsum covered with a dense group of 70–80 very long setae. Anterior part of dorsum covered with short and long spines. Genital plate with a curved row of about 12 spines in each half of the posterior margin. Surface of the genital plate covered with about 100 short spines, some of them on isolated sclerotized bases. Ethiopian (Figs. 36, 37). **Cyclopodia greeffi** Karsch

— As above but only 35–50 long setae on the posterior part of the dorsum and only about 65 spines on the surface of the genital plate. Arabia.
Cyclopodia greeffi arabica Theodor

17. 5–8 short setae at the anterior dorsal margin of the head and between the eyes. Tergite 1 + 2 with a marginal row of 2 longer setae laterally and very short spines in the middle. Posterior part of the surface covered with 5–6 rows of short spines which are longer laterally. **Cyclopodia solomonarum** sp.nov.

— Only 2 setae at the anterior dorsal margin of the head. Marginal row of tergite 1 + 2 with longer setae. Only 1–2 rows of short spines on the surface near the marginal row. 18

18. The marginal row of tergite 1 + 2 consists of setae which are longer laterally and shorter and more closely standing in the middle. Posterior row of tergite 6 with some equally spaced, moderately long setae in the middle, in front of the anal segment (Fig. 47). **Cyclopodia tenuis** Schuurmans Stekhoven & Hardenberg

— The marginal row of tergite 1 + 2 consists of equally spaced setae which are not much shorter in the middle. Posterior row of tergite 6 with 4 long setae in the middle with a space between the 2 median setae (Fig. 48). **Cyclopodia (?) inflatipes** Speiser

19. Small species (2·5 mm.). Dorsum of abdomen covered with short spines in most species. Sternites 5–7 with sclerites. 20

— Larger species (3–4 mm.). Several rows of long setae on the dorsum of the abdomen. Sternites 6 and 7 bearing sclerites. 21

20. Tergite 1 + 2 nearly triangular, with a marginal row of 16–20 setae. Surface bare except for a premarginal row of 4–5 short spines. Sclerites on sternites 5 and 6 standing at right angles, or nearly so, to the median line. Anal sclerite strip-like, with 2 setae at the end (Figs. 54, 55). **Cyclopodia ferrarii** Rondani

— Tergite 1 + 2 elliptical, with a marginal row of 27 setae. A group of about 20 short spines in the middle of the posterior part of the surface. Sclerites on sternites 5 and 6 standing obliquely, at an angle of about 45° to the median line. Anal sclerite elliptical with about 6 short setae (Figs. 61, 62). **Cyclopodia obliqua** sp.nov.

21. Larger species (3·5–4 mm.). 3 groups of long setae, each divided into lateral halves and consisting of 2–3 rows, on the dorsum of the abdomen. The lateral setae of the posterior groups are very long and curved towards the anal segment. A row of short setae anterior to the sclerites on sternite 6. Sternite 7 with a double marginal row of long setae (Figs 71, 72). **Cyclopodia macrura** Speiser

— Smaller species (3–3·5 mm.). The 2 anterior groups of setae on the dorsum consist of single rows of moderately long setae. The posterior groups consist of a single row on the dorsum and of double rows on the pleurae. All setae are straight and not

curved against the anal segment. The row of short setae anterior to the sclerites on sternite 6 is absent. Sternite 7 with a marginal row which is single in the middle and double laterally (Figs. 75, 76). **Cyclopodia orthotricha** sp.nov.

The females of *Cyclopodia truncata* sp.nov., *C. simulans* sp.nov. and *C. brachythrinax* sp.nov. are unknown.

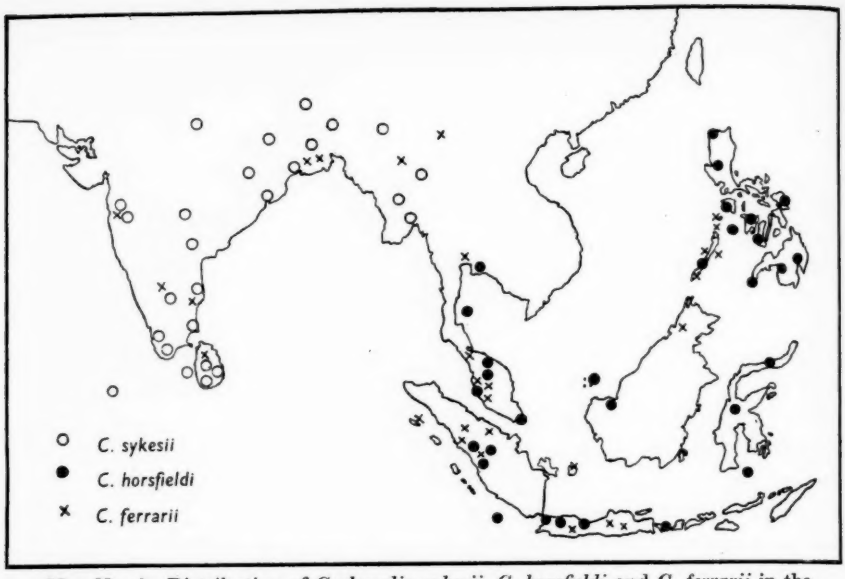
DISTRIBUTION AND HOSTS

Only a few species are known in sufficient numbers so that information about their distribution and hosts can be given. Species of *Cyclopodia* are mainly parasites of Pteropidae and show a more or less pronounced specificity. Thus *Cyclopodia greeffi* is a specific parasite of *Eidolon helvum* and there are only a few records from *Rousettus angolensis* and *Epomophorus* sp. *Cyclopodia sykesii* is mainly known from *Pteropus giganteus* and its subspecies. *C. horsfieldi* has been recorded mainly from *Pteropus vampyrus* and its subspecies and from several other species in the Malaysian region and in the Philippines. *C. albertisi* has been recorded from 3 species of *Pteropus* in Australia and occurs on others in New Guinea and the Islands north and west of it. Several other species with restricted distribution occur on the species of *Pteropus* in their area as indicated in the descriptions. *Cyclopodia pembertoni* has so far been recorded from insectivorous bats only (*Notopteris macdonaldi*), although pteropids occur in its area and are parasitized by *C. inclita*. If it should prove that *C. pembertoni* is a specific parasite of insectivorous bats, this would be the first exception from the rule that the species of *Cyclopodia* are parasites of Pteropidae.

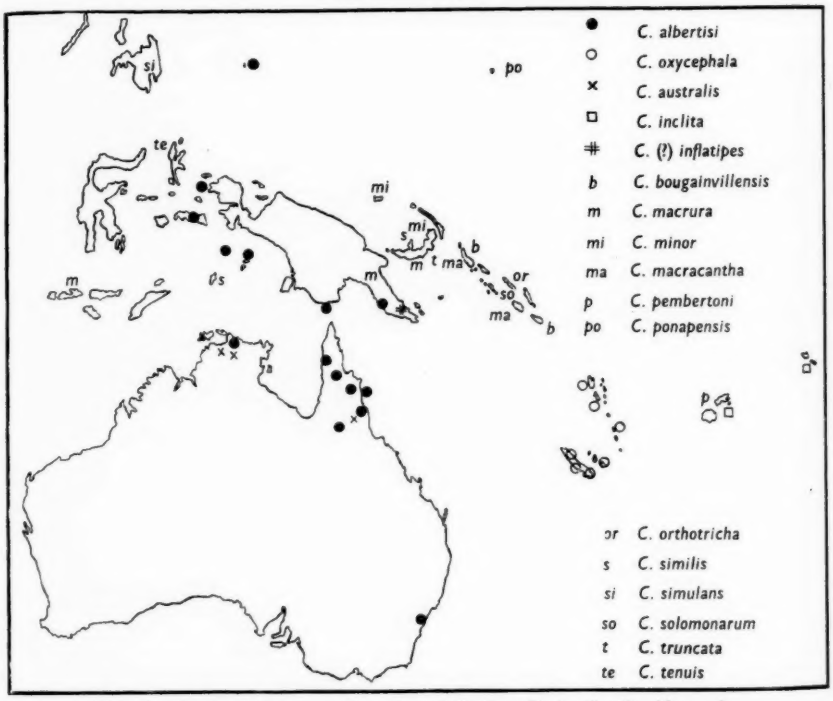
Some species have a wide distribution. *Cyclopodia sykesii* occurs in India over most of the area of distribution of *Pteropus giganteus* except in north-west India. *C. horsfieldi* occurs over most of the Malaysian region, together with *Pteropus vampyrus*, but there are no records from Indochina where *P. vampyrus* occurs. It also occurs in the Philippines on several other species of Pteropidae as indicated in the description (see map no. 1). *Cyclopodia albertisi* has been recorded from north-east Australia, New Guinea and some islands west of New Guinea. It has also been found in Palau Island in the Carolines. *C. ferrarii* has a wide distribution, from southern India and Ceylon along the east coast of the Indian Peninsula to Burma, Malaya, Siam, Sumatra, Borneo, Java and to Palawan in the Philippines where it forms a separate subspecies. It is mainly a parasite of the genus *Cynopterus* and occurs more rarely on related genera (*Chironax, Megaerops*) and there are a few records of stragglers from insectivorous bats. Schuurmans Stekhoven mentions, in addition, *Macroglossus minimus* and *Rousettus amplexicaudatus* as hosts.

Cyclopodia greeffi has a particularly interesting distribution. It occurs in a belt across central Africa between 10° lat. south and 20° lat. north, while its specific host *Eidolon helvum* occurs over nearly the whole of Ethiopian Africa (see map no. 1, Theodor, 1957).

Some of the Pacific species have a restricted distribution (Map no. 2). *Cyclopodia oxycephala* is known only from the New Hebrides and New Caledonia and *C. inclita* from Fiji and Samoa. Several species are known from the type localities



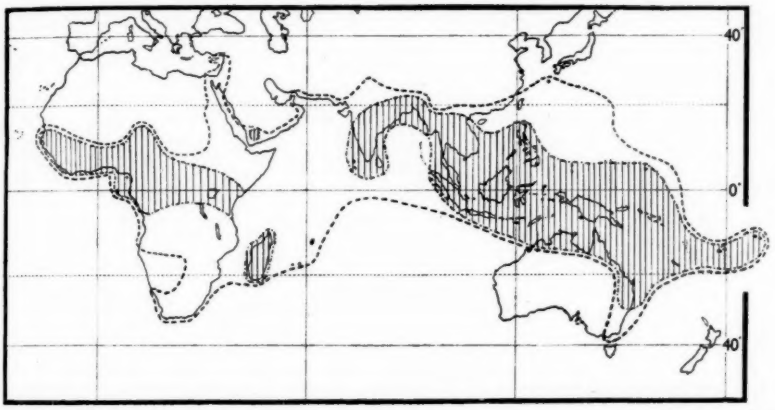
Map No. 1. Distribution of *Cyclopodia sykesii*, *C. horsfieldi* and *C. ferrarii* in the Oriental Region.



Map No. 2. Distribution of species of *Cyclopodia* in the Pacific region.

only. The distribution of some species will probably prove wider when more material will be available. This concerns particularly the area north of New Guinea where very little collecting has been done. *C. macrura* is known from three localities, Sumbawa, New Guinea and New Britain, and a closely related species which may eventually prove to be a subspecies of *C. macrura*, *C. orthotricha*, occurs in the Solomon Islands.

The centre of development of the genus seems to lie in the area including New Guinea, north-east Australia, the Bismarck Archipelago and the Solomon Islands. 11 species occur in this area. Only 3 species occur farther east, in New Caledonia,



Map. No. 3. Distribution of the family Pteropidae (broken line), after Eisentraut, and of the genus *Cyclopodia* (shaded area).

the New Hebrides, the Fiji Islands and Samoa, which is also the eastern limit of the distribution of the Pteropidae. 7 species occur in the Oriental region of which only two enter the Indian Peninsula. Only 2 species occur in the Ethiopian region. The known distribution of the genus *Cyclopodia* thus coincides closely with that of the Pteropidae, except that it is more restricted towards the northern and southern limits of the area. This is particularly marked in the Ethiopian region where the *Cyclopodias* do not follow their hosts into the Middle East and into South Africa. (The record of *C. sykesii* from Judaea is almost certainly due to an error in labelling.) The same applies to Micronesia from where Pteropids are recorded much farther north than species of *Cyclopodia* (Map no. 3).

REFERENCES

Bigot, J. (1860). Diptères exotiques nouveaux. *Ann. Soc. Ent. Fr.* **3**, ser. **8**, 219.

Eisentraut, M. (1944). Biologie der Flederhunde (Megachiroptera). *Biol. gener.* **18**, 327.

Ellerman, J. R. & Morrison-Scott, T. C. S. (1951). *Checklist of Palaearctic and Indian Mammals.* British Museum (Nat. Hist).

Falcoz, L. (1923). Diptères Pupipares de la Nouvelle-Caledonie et des Iles Loyalty (Streblidae et Nycteribiidae). In F. Sarasin & J. Roux, *Nova Caledonia, Zoologie*, vol. III, p. 83. Berlin: C. W. Kriedel's Verlag.

Ferris, G. F. (1925). Third report upon Diptera Pupipara from the Philippine Islands. *Philipp. J. Sci.* **27**, 413.

JOBLING, B. (1928). The structure of the head and mouth-parts in the Nycteribiidae (Diptera Pupipara). *Parasitology*, **20**, 254.

KARAMAN, Z. (1939). Beitrag zur Kenntnis der Nycteribien. II. *Ann. Mus. Serb. merid.* **1**, 31.

KARAMAN, Z. (1948). Beitrag zur Kenntnis der Nycteribien. IV. *Rad. Ac. Jugoslav.* vol. 273, 117.

KARSCH, F. (1884). *S.B. Ver. Befoerd. ges. Naturw. Marburg*, p. 77.

KISHIDA, K. (1932). *Iconographia Insectorum Japonicorum*. Tokyo.

KOLENATI, F. A. (1863). Beitraege zur Kenntnis der Phthirio-Myiarien. *Hor. Soc. Ent. Ross.* **2**, 9.

LAURIE, E. M. O. & HILL, J. E. (1954). List of land mammals of New Guinea, Celebes and adjacent islands. (Brit. Mus. Nat. Hist.)

MEIJERE, J. C. H. de (1899). *Cyclopodia horsfieldi* n.sp., eine neue Nycteribie aus Java. *Tijdschr. Ent.* **42**, 153.

MUKERJI, D. & DASGUPTA, B. (1954). Some aspects of the anatomy of the bat-fly, *Cyclopodia sykesii* Westw. *Proc. Soc. Zool.* **7**, (1) 1.

RONDANI, C. (1878). Muscaria exotica Musei civici Januensis observata et distincta. Frag. IV. Hippoboscita exotica non vel minus cognita. *Ann. Mus. Civ. Stor. Nat. Genova*, **12**, 150.

SCHUURMANS STEKHOVEN, J. H. & HARDENBERG, D. J. F. (1938). Fledermausfliegen aus Niederlaendisch Ost-Indien. *Capita zool.* **8**, 1.

SCOTT, H. (1908). On certain Nycteribiidae with descriptions of two new species from Formosa. *Trans. Ent. Soc. Lond.* 359.

SCOTT, H. (1914). On some Oriental Nycteribiidae. *Ann. Mag. Nat. Hist.* (8), **14**, 209.

SCOTT, H. (1917). Notes on Nycteribiidae with descriptions of new species. *Parasitology*, **9**, 593.

SCOTT, H. (1932). Some Nycteribiidae from the Australian Region. *Stylops*, **1**, 16, 25.

SPEISER, P. (1901). Ueber die Nycteribien. *Arch. Naturgesch.* **67**, 11.

THEODOR, O. (1953). On the structure of the genitalia in the Nycteribiidae. *Trans. IX. Int. Congr. Ent. Amsterdam*, vol. II, 27.

THEODOR, O. (1954). Nycteribiidae, 66a. In Lindner, E., *Die Fliegen der Palaearktischen Region*. Stuttgart.

THEODOR, O. (1957). The Nycteribiidae of the Ethiopian Region and Madagascar. *Parasitology*, **47**, 457.

THOMPSON, G. B. (1937). A list of the Diptera (Nycteribiidae and Streblidae) recorded from bats of the Pacific Islands. *Ent. Mon. Mag.* **73**, 202.

WESTWOOD, J. O. (1835). On Nycteribia, a genus of wingless insects. *Trans. Zool. Soc. Lond.* **1**, 275.

(*MS. received for publication* 21. v. 1958.—Ed.)